EXPLORATION OF NEMATOPHAGOUS AND MYCOPARASITIC POTENTIAL OF NATIVE STRAINS OF NEMATODE TRAPPING FUNGI OF BUNDELKHAND REGION AGAINST SOME PLANT PATHOGENS

THESIS

SUBMITTED TO THE

BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, BANDA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE (AGRICULTURE)

IN

PLANT PATHOLOGY

BY

Mr. ABHAY PRATAP SINGH

(ID. NO. 2035)

DEPARTMENT OF PLANT PATHOLOGY

(COLLEGE OF AGRICULTURE)

BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY,

BANDA-210001, UTTAR PRADESH, INDIA

2023

Dedicated

To
my beloved
Grand Father

Shri Amar Singh

Abhay ....

ACKNOWLEDGEMENT

*With all humility & sincerity, I own my submission and reverence to "**Lord Krishna",** as it all happened by his grace, who blessed me with strength and zeal to accomplish this important task of life & achieve this milestone.*

*I find no words to express my sincere feelings of gratitude towards my guide and Chairman of advisory committee **Dr. Dharmendra Kumar,** Professor & head Department of Plant Pathology, Banda University of Agriculture and Technology, Banda. For his guidance, keen interest, critical evaluation and appreciation in the execution of my entire work, meticulous supervision, and constant encouragement during whole degree programme. I consider myself fortunate to have worked under his proficient supervision and guidance. I was greatly benefitted by his excellent academic qualities and his moral support offered by him to me from time to time during the period.*

*I extend my sincere gratitude to other members of my advisory committee **Dr. Sunil Kumar** Assistant Professor, Department of Plant Protection, College of Horticulture,BUAT, Banda. **Dr. Rakesh Pandey** Professor, Department of Entomology, College of Agriculture, BUAT, Banda. **Dr. C.M. Singh,** Assistant Professor, Department of Genetics and Plant Breeding, College of Agriculture, BUAT, Banda for their valuable comments, helpful suggestions and support in completion of the present study.*

*I am very much thankful to **Dr. Narendra Pratap Singh**, Hon'ble Vice- Chancellor**, Dr. Mukul Kumar,** Dean PGS, College of Agriculture, **Dr. G.S.Panwar,** Dean, College of Agriculture, BUAT, Banda for providing me all necessary facilities during the research work.*

*At the outset, I intend to place on record my deep sense of gratitude & respect to **Dr. Vivek Singh,** Assistant Professor, Department of Plant pathology, **Dr. H.S. Negi**, Assistant Professor, Department of Plant Pathology, **Dr. V.K. Singh**, Professor,Department of Plant Pathology, **Dr. Gaurav Shukla**, Assistant Professor, Department of Statistics, and staff member of the department **Shri Ashok Kumar, Mr. Satya Prakash** for their help, constant encouragement and suggestions at various stages during research work.*

*I would like to extend my profound thanks to my senior **Mr Arvind Kumar, Mr. RishinathPandey, Mr. Pradeep Kumar, Mr. Hariom Dwivedi, Mr. Radhe Krishan, Mr. Amit Kumar, Mr.Grijesh Kumar, Mr. Utkarsh Upadhyay, Mr Ajay Kumar, Mr.***

Neelesh Shahani, Mr. RohitKumar, Ms. Sakshi Pandey *my heartiest gratitude toward the help rendered by my close friends* ***Prashant Kumar Tripathi, Sunil Kumar Varma, Vijay Kumar Kushwaha, Atul Singh, Surjeet,Shravan Kumar, Ujjawal Rai, Deepak Tomar*** *for providing kind help and fruitful suggestions,whose love, affection, inspiration and good wishes have always with me.*

I must not miss this opportunity to express my special thanks to my batch mates ***Ms. Pooja Yadav, Mr. Shrikant Rawat, Mr. Ali Khan and Ms. Anchal Singh****, for their cooperation, help, moral support and encouragement during the entire period of study. I wish to record special thanks to my lovely juniors* ***Rajan yadav, Yogendra Kumar, Yogendra Rajput, Sanjay Yadav, Ram Prakash, Vishal, Roma and Shweta,*** *for their love, affection, moral support, care and encouragement.*

Next to God the parents" I have no words to express renunciation, heartfelt gratitude to my father ***Shri Vijay Singh*** *for his salient wishes and blessing, my mother* ***Smt. Beenu Yadav,*** *for devotion, sacrifice, love, and affection which makes me what I am and brings me to this level to do the goodand great deeds. I express thanks to my younger sister* ***Ankita Yadav.***

I am also thankful to laboratory staff of Department of Plant Pathology, and Department of Genetics and Plant Breeding for providing facilities during the course of study and research work.

Abhay Pratap Singh

(Mr. Abhay Pratap Singh)

Place: BUAT, Banda

Date: 09-8-2023

Dr. Dharmendra Kumar
Professor & Head
Plant Pathology

Department of Plant Pathology
College of Agriculture
Banda University of Agriculture
& Technology, Banda-210001

Dated - 09-8-2023

Certificate I

This is to certify that the thesis entitled **"Exploration of nematophagous and mycoparasitc potential of native strains of nematode trapping fungi of Bundelkhand region against some plant pathogens"** submitted in partial fulfillment of the requirement for award of the degree of **Master of Science (Agriculture)** in **Plant pathology, College of Agriculture,** Banda Universityof Agriculture & Technology (Banda), is a genuine record of bonafide research work carried out by **Mr. Abhay Pratap Singh, Id. No. 2035** under my guidance and supervision. The results of the investigation in this have not so far been submitted for any other degree or diploma.

It is further certified that the help or information received during the course of investigation and preparation of the thesis have been duly acknowledged.

09-8-2023
Dr. Dharmendra Kumar
Chairman
(Advisory Committee)
(Dr. Dharmendra Kumar)
Professor & Head
Deptt. of Plant Pathology
B.U.A.T., Banda-210001 (U.P.)

Certificate – II

We, the undersigned members of the Advisory Committee of **Mr. Abhay Pratap Singh, Id. No. 2035** a candidate for the degree of **Master of Science (Agriculture)** in **Plant Pathology** have gone through the thesis manuscript and agreed that the thesis entitled **"Exploration of nematophagous and mycoparasitc potential of native strains of nematode trapping fungi ofBundelkhand region against some plant pathogens"** may be submitted in the partial fulfillment for award of the degree.

09-8-2023

(Dr. Dharmendra Kumar)
Professor & Head
Department of Plant Pathology
Chairman
Advisory Committee

Endorsed by:

09/08/2023

(Dr. Sunil Kumar)
Assistant Professor
Department of Plant Protection
Co- Advisor

09.08.23

(Dr. Rakesh Pandey)
Professor
Department of Entomology
Co - Advisor

(Dr. C.M. Singh)
Assistant Professor
Department of Genetics and Plant Breeding
Co- Advisor

09-08-2023

(Head)
Department of Plant Pathology
(Dr. Dharmendra Kumar)
Professor & Head
Deptt. of Plant Pathology
B.U.A.T., Banda-210001 (U.P.)

Certificate – III

This is to certify that the thesis entitled **"Exploration of nematophagous and mycoparasitc potential of native strains of nematode trapping fungi of Bundelkhand region against some plant pathogens"** submitted by **Mr. Abhay Pratap Singh, Id. No. 2035** in partial fulfillment of the requirement for award of the degree of **Master of Science (Agriculture) in Plant Pathology, College of Agriculture,** Banda University of Agriculture & Technology (Banda) was examined and approved on 21/08/2023 at 9.45-10.45 am.

21-08.2023

(Dr. Dharmendra Kumar)
Professor & Head
Department of Plant Pathology
Chairman
Advisory Committee

Dr. S. S. Vaish
Professor
Department of Mycology & Plant Pathology
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi-221005, India
External Examiner

Endorsed by:

21/08/2023

(Dr. Sunil Kumar)
Assistant Professor
Department of Plant Protection
Co- Advisor

(Dr. Rakesh Pandey)
Professor
Department of Entomology
Co- Advisor

(Dr. C.M. Singh)
Assistant Professor
Department of Genetics and Plant Breeding
Co- Advisor

21-8-2023

Head
Department of Plant Pathology
(Dr. Dharmendra Kumar)
Professor & Head
Deptt. of Plant Pathology
B.U.A.T., Banda-210001 (U.P.)

Dean College of Agriculture

Dean Post Graduate Studies

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF LINE GRAPHS

LIST OF APPENDIXES

LIST OF ABBREVIATIONS

pH	:	Power of hydrogen
psi	:	Pounds per square inch
Spp.	:	Species
Microbes	:	Microorganisms
100x	:	100 times magnification
%	:	Per cent
Min	:	Minute
CMA	:	Corn meal agar
RDA	:	Rabbit Dung Agar
CRD	:	Completely randomized design
µl	:	Microliter
±	:	Plus, or minus
µm	:	Micrometer
^{0}	:	Degree
C	:	Centigrade
et al.	:	and others
Fig.	:	Figure
g	:	Grams
i.e.,	:	That is
Hrs.	:	Hours
mm	:	Millimetre
ml	:	Millilitre
no.	:	Number
viz.	:	Namely
l	:	Liter
Sq.	:	Square
bp	:	Base pair
CTAB	:	Cetyl trimethyl Ammonium Bromide
DNA	:	Deoxyribonucleic Acid
EDTA	:	Ethylene diamine tetra acetic acid
NaCl	:	Sodium Chloride
ng/µl	:	Nano gram per micro litre
nm	:	Nano meter
PCR	:	Polymerase Chain Reaction
TAE	:	Tris Ammonium Acetate EDTA

ABSTRACT

Name	Abhay Pratap Singh		
Semester	IV	**ID. No.**	2035
Year of Admission	2021	**Degree**	M.Sc. (Agriculture)
		Department	Plant Pathology
Major	Plant Pathology		
Thesis Tittle	Exploration of nematophagous and mycoparasitc potential of native strains of nematode trapping fungi of Bundelkhand region against some plant pathogens.	**Minor**	Entomology

Nematode trapping fungi are known to be nematophagous and mycoparasitic and widely distributed in agricultural and horticultural soil. In present investigation seven spp. of the nematode-trapping fungi were isolated from Bundelkhand region of Uttar Pradesh and their mycoparasitic and nematophagous ability were tested for exploration of their potential use in agriculture. These nematode trapping fungi were characterized and identified as *Arthrobotrysoligospora* isolate 1, *Arthrobotrys oligospora* isolate 2, *Arthrobotrys thaumasia* isolate 1, *Arthrobotrys thaumasia* isolate 2, *Arthrobotrys musiformis, Arthrobotrys conoides, Monacrosporium sphaeroides, Dactylellina phymatopaga* and *Drechslerella brochopaga.* Exploration of their mycoparasitc activity against *Rhizoctonia solani* (AG-1&AG-3) indicates that five species are potential mycoparasite of *Rhizoctonia solani* (AG-1&AG-3). Out of the five mycoparasitic nematode trapping fungi *Arthrobotrys thaumasia* isolate 1 and *Arthrobotrys thaumasia* isolate 2 were found most parasitic to *Rhizoctonia solani* (AG-1&AG-3) followed by *Arthrobotrys musiformis Arthrobotrys oligospora* isolate 2, *Arthrobotrys oligospora* isolate 1,*Monacrosporium sphaeroides,* and *Arthrobotrys conoides.* Exploration of nematophagous ability against *Anguina triticii* and *Meloidogyne javanica* shows that all fungi were able to capture and kill the *Anguina triticii* and *Meloidogyne javanica* by different type of trapping organ. *Arthrobotrys oligospora* isolate 2 was found highly nematophagous against *Anguina triticii* whereas *Drechslerella brochopaga* was found highly nematophagous against *Meloidegyne javanica* followed by *Dectylellina phymatopaga.* During present investigation it was found that *Arthrobotrys thaumasia, Arthrobotrys oligospora, Arthrobotrys musiformis, Monacrosporium sphaeroides,* and *Arthrobotrys conoides* could be used as biocontrol agent for *Rhizoctonia solani* (AG-1, AG-3 *Anguina triticii,* and *Meloidogyne javanica*) whereas *Drechslerella brochopaga* and *Dactylellina phymatopaga* could be used as biocontrol agent of *Meloidogyne javanica* and *Anguina triticii.*

09.8.2023

(Dr. Dharmendra Kumar)
Major advisor

(Abhay Pratap Singh)
Student

सारांश

नाम	अभय प्रताप सिंह	**आई0डी.नं0**	2035
सेमेस्टर	IV	**उपाधि**	एम. एस–सी, (कृषि) पादप रोग विज्ञान
प्रवेश का वर्ष	2021	**विभाग**	पादप रोग विज्ञान
प्रमुख विषय	पादप रोग विज्ञान	**सहायक विषय**	कीट विज्ञान

शोध का शीर्षक– बुन्देलखण्ड क्षेत्र के कुछ पौध रोग जनको के खिलाफ सूत्रकृमि भक्षक कवको के मूल उपप्रजातियों की सूत्रकृमि एवं कवक भक्षण क्षमता की अन्वेषण

सूत्रकृमि भक्षक कवक उन मृदाओं में बहुतायत से पाये जाते है जहाँ मृदा में कार्बनिक पदार्थो की मात्रा अधिक होती है। यह कवक प्रजातियाँ मृदा में उपस्थित पादप परजीवी सूत्रकृमियों एवं *राइजोक्टोनिया सोलेनाई* प्रजाति की फंफूद के लिए परीजीविता द्वारा संक्रमण करके इन रोग जनको की मात्रा को न्यूनतम करती है। इस शोध प्रबन्ध के अर्न्तगत बुन्देलखण्ड की मृदा मे पाये जाने वाले सूत्र कृमि भक्षक कवकों की जैविक नियंत्रण क्षमता का पता लगाने के लिये इनको मृदा से पृथक कर इनकी पहचान की गई। *आर्थोबोट्रिस म्यूसिफार्मिस, आर्थोबोट्रिस ओलिगोस्पोरा, आर्थोबोट्रिस थॉमेसिया, आर्थोबोट्रिस कोनॉयडिस, ड्रेसलेरेल्ला ब्रोकोपेजा, मोनाक्रोस्पोरियम स्फेरॉयडिस* और *डेक्टिलेलिना फाइमेटोपेजा* नामक सात प्रजातियों एवं इन कवकों की चार विभेदों का पृथक्करण करके इनकी पहचान की गयी। अध्ययन में यह पाया कि सात में से पांच प्रजातियाँ राइजोक्टोनिया सोलेनाई के कवक तन्तु को संक्रमित करने में सक्षम है तथा दो प्रजातियां *ड्रेसलेरेल्ला ब्रोकोपेजा* तथा *डेक्टिलेलिना फाइमेटोपेजा, राइजोक्टोनिया सोलेनाई* में सक्रमण करने में असफल रहीं। सभी प्रजातियां *एंग्विना ट्रिटिसाई* एवं *मेलायडोगाइन जावानिका* नामक पादप परजीवी सूत्रकृमियों का भक्षण करने में सफल पायी गयी। *आर्थोबोट्रिस ओलिगोस्पोरा* द्वारा *एंग्विना ट्रिटिसाई* का सर्वाधिक भक्षण किया गया, जबकि *ड्रेसलेरेल्ला ब्रोकोपेजा* द्वारा *मेलायडोगाइन जावानिका* का सर्वाधिक भक्षण किया गया। अध्ययन से यह ज्ञात किया कि *आर्थोबोट्रिस म्यूसिफार्मिस, आर्थोबोट्रिस ओलिगोस्पोरा, आर्थोबोट्रिस थॉमेसिया, आर्थोबोट्रिस कोनॉयडिस एवं मोनाक्रोस्पोरियम स्फेरॉयडिस* सूत्रकृमियों के भक्षण के साथ–साथ राइजोक्टोनिया सोलेनाई के कवक जाल को संक्रमित करने में सफल पायी गईं, जिसका उपयोग पादप परजीवी सूत्रकृमि एवं राइजोक्टोनिया सोलेनाई के जैव नियंत्रक के रूप मे किया जा सकता है। *ड्रेसलेरेल्ला ब्रोकोपेजा एवं डेक्टिलेलिना फाइमेटोपेजा* का उपयोग *एंग्विना ट्रिटिसाई एवं मेलायडोगाइन जावानिका* के जैविक नियंत्रण मे किया जा सकता है।

09-8-2023

डा0 धर्मेन्द्र कुमार
मुख्य सलाहकार

अभय प्रताप सिंह
छात्र

Chapter-I

INTRODUCTION

Plant parasitic nematodes and *Rhizoctonia solani* are the most important pathogens ofagricultural and horticulture crops, generating massive economic losses across the world. On aglobal scale, plant-parasitic nematodes cause an average loss of 12.3% each year in 40 important crops; the losses are higher in developing countries (14.6%) than in industrialized countries (8.8%) (Elling, 2013). Plant-parasitic nematode infection is projected to cause an annual loss to Indian agriculture of over 210 crores of rupees (Jain *et al*., 2007). *Meloidogyne spp.* are among the many species of plant parasitic nematodes that are known to infect 350 plant species in India. Singh (1972), was the first to report the root-knot disease caused by *M. incognita* and *M. javanica* on mungbean in India. Mungbean yield loss of 28% was observedin a field infested with a combined population of *M. incognita* and *R. reniformis* (Castillo etal., 1977). Under field circumstances, avoidable losses due to *M. javanica* in mungbeanranged from 42.1 to 93.4% (Gupta and Verma, 1990). Similarly, in Uttar Pradesh, yield losses of 18 to 65% owing to *M. incognita* and 23 to 49% due to *M. javanica* on mungbean have been confirmed (Sharma et al., 2000). Mungbean infected with *M. incognita* showed a considerable decrease in plant growth, nodulation, and nitrogen content of the shoot and root (Hussaini and Seshadri, 1975; Inderjit Singh et al., 1977). In order to crop destruction, the damage brought out by the root-knot nematode *M. incognita* have been substantially greater than other plant-parasitic nematodes (Khushbu, 2016).

Root-knot nematode infections cause significant morphological and physiological symptoms in plant roots. Root-knot nematodes hatch from eggs and enter the roots of their hosts as second-stage juveniles. Large galls or knots produced by hypertrophy and hyperplasia are developed throughout the root system of afflicted plants as a result of nematode penetration and infection. The stationary endoparasite root-knot nematodes eventually give birth to females in the roots of their host (Starr *et al.,* 1993; Ogbuji, 2004 and Anwar *et al*., 2007). One of the most common pathogens producing yellow ear disease or ear cockle, in which the grains turn into cockles, is *Anguina triticii*. *A. tritici* often attacks wheat, rye, and barley over the world, while barley is rarely affected in India. (Paruthi and Gupta, 1987). The yield loss is more when the nematode was found associated with yellow ear rot causing bacterium on wheat (Maqbool, 1988). In wheat, losses up to 100% have been reported by

Paruthi et al. (1987), 90% by Reddy (1983), up to 52% by Paruthi and Bhatti (1988) and 50 and 65% losses in wheat and rye respectively by Leukel (1957). In terms of money, losses worth Rs. 6.54 million have been reported in India (Sakhuja et al., 1990). *A. tritici* is a sizable nematode that ranges in length from 2 to 2.55 mm. When it enters the floral primordia, it changes from being an ectoparasite to being an endoparasite. The galls are where the nematode survives. The galls soften in the presence of the right moisture and temperature; infectious juveniles (J2) of the second stage emerge and swim upward in a thin film of water. On wheat, it results in plant stunting, leaf deformation, wrinkling, and twisting. Galls, which contain a desiccated mass of nematodes, are created from seeds. Galls are smaller, lighter, and range in colour from light brown to black when compared to regular wheat seeds.

Around 250 host plant species from the *Poaceae, Fabaceae, Solanaceae, Amaranthaceae, Brassicaceae, Rubiaceae, Malvaceae, Asteraceae, Araceae, Moraceae,* and *Linaceae* families are susceptible to *Rhizoctonia solani* anastomosis group AG1 and AG3 infection (Chahal *et al.*, 2003). Several studies, including the root rot disease complex inducedby *R. solani* and *M. incognita* on green beans, suggested that *Rhizoctonia*- root rot was more severe in the presence of root- knot nematodes. (France and Abhawi 1994; Mokbel *et al.* 2007 etc.).

Rhizoctonia solani is a necrotropic Basidiomycetous fungus, known for its highly destructive lifestyle (Sinclair 1970; Anderson 1982; Gonzalez Garcia *et al.,* 2006). Necrotrophs are unique pathogens which penetrate and kill host cell by secretion of cell wall degrading enzymes and toxins and then derive the nutrients released from dead host tissue. *R. solani* has wide host range including species in *Asteraceae, Brassicaceae, Fabaceae, Solanaceae* and as well as some ornamental plants and forest trees (Lee and Rush 1983; Rush and Lee 1992; Pan *et al.,* 1999; Zou *et al.,* 2000). Based upon hyphal fusion affinity (known as anastomosis) isolates of *Rhizoctonia solani* has been classified into 14 anastomosis groups, namely AG-1 to AG-13 and AG-BI (Ogoshi 1987; Guil lemaut *et al.,* 2003; Gonzalez Garcia*et al.,* 2006; Taheri *et al.,* 2007). Based on characteristic features like colony morphology, biochemical genetic and pathogenicity, *Rhizoctonia solani* AG-1 has been further divided into six intraspecific groups i.e IA, IB, IC, ID, IE and IF (Ogoshi 1987; Yang and Li 2012; Wibber *et al.,* 2013;). AG-1 IA causes sheath blight disease in rice. Several workers have reported thatsclerotia are first grey white, later brown to black in color, sub-globose slightly flattened in shape and vary from 0.5-5.0 mm in size (Matsumoto

and Yamato, 1935; Ryker, 1939; and Palo, 1926).

The perfect state, *Thanatephorus cucumeris* is known for various names and includes familiar names like *Hypschnus cucumeris*, *Hypschnus solani*, *Corticium solani*, *Corticium microsclerotia* (*Rhizoctonia microsclerotia*) and *Pellicularia filamentosa*. Other possible synonyms are *Corticinum sasaki* and *Corticium aerolatum*, *Thanetephorus particolus* (Kotila) Flintje which were earlier considered as distinct species have also been included in *Thanatephorus cucumeris* by Talbot (1965). The perfect state of *Rhizoctonia solani* is considered to be *Thanatephorus cucumeris* (Frank).

In Japan, sheath blight on rice was first noted in 1910. The disease was recognised byseveral names as it spread to other Asian nations, including "Oriental leaf and sheath blight," "Sheath blight," "Pellicularia sheath blight," "Sclerotial blight," and "Banded blight of rice." (Srinivasachary Willocquet and Savary 2011). In India, the disease was first reported from Gurudaspur, Punjab (Paracer and Chahal, 1963) and later it was reported from Uttar Pradesh (Kohli, 1966). The pathogen causes lesions on the sheath, affecting grain filling and total yieldin rice (Wu et al. 2012). *Rhizoctonia solani*, the causative agent of sheath blight of rice, is responsible for yield loss up to 45% (Margani and Widadi (2018).

The web blight of mung beans, which was initially noticed in Philippine is also known to be caused by *Rhizoctonia solani*. (Nacien, 1924). In India, web blight diseaseon mung bean was reported from Kanpur, Uttar Pradesh (Dwivedi and Saksena 1974). This disease has also been reported from Punjab, Assam, Madhya Pradesh, Bihar, Haryana, Rajasthan, Jammu & Kashmir and Himanchal Pradesh. In India, the infection significantly reduces mung bean output. It was shown to diminish grain output by 33 to 40% (Singh et al., 2013). Different types of symptoms are appeared at different stages of plant.

At the seedling stage: On the collar region, an uneven, reddish-brown lesion developed. As the lesion became larger over time, the seedlings eventually died.

On leaves: A tiny, erratic water-soaked patch started to form on the surface of leaves two months after the seeding. At high humidity the spots enlarged which were surrounded by water-soaked areas. The leaves also include white mycelia, which eventually developed into sclerotia that are brown to black in coloration.

On other parts: The majority of the plant parts including the leaves, stems, petioles, and pods, were affected by the lesions. Whole plants were reported to be blighted a short time after symptoms first appeared.

R. solani and plant parasitic nematodes are reported to last longer in soil. The management of *R. solani* and plant parasitic nematodes is extremely challenging in the soil just as other soil-borne infections. Crop rotation, summer ploughing, and floods had little of an effect on the inoculum of *R. solani* and plant parasitic nematodes in the soil. Despite the fact that pesticides have been shown to reduce plant diseases, widespread use of pesticides hasresulted in ground water contamination, toxicity to mammals and birds, and residues in food. As a result, several of the most efficient chemicals have been deregistered. Therefore, the needof the hour is to look for non-chemical means of controlling plant diseases. To handle these economically significant infections, it is thus crucial to look for alternative control measures that are safe for the environment, non-hazardous, and ecologically friendly. Application of or cultivation of fungi antagonistic to plant diseases is one potential non-chemical strategy for management of plant infections.

The term mycoparasitism is commonly used to indicate the interrelationships whereby a fungus parasitizes on another fungal host. Mycoparasitism can be dated to at least 400 million years ago by fossil evidence (Taylor et al., 2005). Fungi adopting parasitic life style on the hyphae of other fungi through phenomenon called mycoparasitism are of agricultural importance when the parasitized fungal host is a plant pathogen. In recent past, many mycoparasitic fungi has been discovered and they have been used as new tool for the bio-control of plant pathogens. The application of some fungi that parasitize the *R. solani* could be a promising alternative for management of sheath blight of rice. The hyphal contact between a mycoparasite and its live host that leads to mycoparasitism is most important to know the ability of mycoparasitic fungi in the lab condition so it should be studied to choose the best bio-control agents. Direct confrontation studies between a mycoparasite and its living host may indicate the mycoparasitic ability of mycoparasite for use of these agents against pathogens for disease management.

The term "biological control of nematodes" refers to the management of nematodes that is brought about by the interaction between soil microorganisms and the soil microfauna, which is accomplished by processes such as parasitism, predation, competition, and antibiosis. Numerous species have been discovered to parasitize or feast upon nematodes, although bacterial and fungal antagonists have garnered more attention and have

been utilised more frequently in worm control. (Krishanppa and Shreenivasa, 2009).

A type of soil-dwelling fungus known as nematode-trapping fungi produce sticky or mechanical traps that trap, kill, and digest motile nematodes (Drechsler, 1937; Drechsler,1941; Pramer, 1964; Thorn and Barron, 1984; Barron, 1977). They produce a variety of morphologically distinct trapping structures, including constricting rings, adhesive knobs, sticky nets, adhesive branches, and non-constricting rings. These fungi produce special structure on their hyphae that allow nematode to be mechanically or adhesionally trapped. Studying nematode-trapping fungus is interesting because they might be used to biologically manage nematodes that parasitize plants and animals. (Kerry, 2000; Larson, 2000). In avariety of soil conditions, nematode-trapping fungus may be found living saprophytically and acting as facultative predators of nematodes (Pramer, 1964., Nordbring-Hertz et al., 2006). These fungi create traps on hyphae on or inside of which nematode can be caught mechanically or by adhesion in order to transition to a predatory lifestyle. Alternately, traps may develop immediately after conidial germination without a hyphal phase in between(Persmark and Nordbring Hertz, 1997) or on short spore germlings (Cook, 1964; Kumar, 2003) so-called conidial traps. These conidial traps are induced in response to cow dung (Dackman and Nordbring-Hertz, 1992), rhizosphere soil (Mankau, 1962; Pressmark and Nordbring-Hertz, 1997) and are compound released by soil nematodes (Kumar, 2003). When nematode-trapping fungus form conidial traps, they may have the chance to catch and extract the nematode's body's rich nourishment for their own growth and development in the soil despite fierce competition from other fungi for resources. Plant health has been improved by using nematode-trapping fungus formulations to control root-knot nematodes in soil (Stirling and Smith, 1998; Stirling et al., 1998; Kumar and Singh, 2006a; Singh et al., 2007; Singh etal., 2012), enhancing the accumulation of defense related biomolecules and induce systemic resistance in plants (Singh et al., 2013). The effectiveness of nematode-trapping fungus can be affected by a number of elements in soil, which is a complex system for maintaining life. For instance, soil fungistasis, which prevents the majority of fungal propagules from germinating (Dobbs and Honson, 1953). Unfortunately, most of the nematode- trapping fungi fail to germinate in soil due to soil fungistasis (Cook, 1964; Mankau, 1962) and thus reduce the nematode killing ability as expected. Introduction of nematode-trapping fungi to soil in the form of spores seems to be most feasible method for large-scale application of such organisms (Mankau, 1962).

Despite the large morphological variation in trapping structures, phylogenies inferred from molecular data have shown that a majority of nematode-trapping fungi belong to a monophyletic group placed in the family of *Orbiliales*, Ascomycota. The role of native strains of nematode-trapping fungi for the biological control of plant-parasitic nematodes and *Rhizoctonia solani* AG1 & AG3 have not been studied in detail. Therefore, keeping in view the importance of nematode- trapping fungi in agriculture and seriousness of losses caused by plant parasitic nematodes and *Rhizoctonia solani* in different agricultural and horticultural crops, the present investigation was undertaken with following objectives.

1. Morphological and molecular characterization of native strains of nematode-trapping fungi.
2. Assessment of nematophagous and mycoparasitic potential of native strains ofnematode-trapping fungi against some plant pathogens.

REVIEW OF LITERATURE

2.1 Historical development of Nematode Trapping fungi

Fresenius' initially described *Arthrobotrys oligospora* as a ubiquitous soil dweller in 1852. Woronin (1870) reported that *A. oligospora* forms hyphal nets, but he was unable to determine their use. Wilhelm Friedrich Zopf (1888) stated that the migrating nematodes are caught in the *Arthrobotrys oligospora* hyphal nets. Therefore, Zoph was the first researcher to identify the biological link between a nematode and a fungus that traps nematodes.

Charles Drechsler's research (1933, 1933a), investigated that nematode-trapping fungi's hyphal nets produce an adhesive substance that captures nematodes. Drechsler's research on nematode-trapping fungus between 1933 and 1975 inspired other scientists to study these organisms for the biological control of both animal and plant parasitic nematodes.

Duddington studied nematode-trapping fungus between 1952 and 1972 and came to the conclusion that they are frequently present in both natural and agricultural soils as well as all kinds of decaying manures. He also made available instructions for using nematode-trapping fungus (Duddington, 1955). His book "The Friendly Fungus" explores a range of subjects pertaining to nematode-trapping fungus (Duddington, 1957). Many scientists have contributed to our awareness of these nematode-trapping fungus. (Juniper,1957; Cooke 1962a; Cooke, 1962b; Cooke, 1963; Higgins, 1967; Barron, 1969; Barron, 1977; Mankau and Clark, 1959; Monoson and Ranieri, 1972; Pramer, 1964; Nordbring-Hertz *et al*., 1977; Stirling, 1979; Jansson and Nordbring-Hertz, 1980).

In India, some early research on nematode-trapping fungus was started by Das Gupta *et al.* (1964), Sachchidananda (1965), Sachchidananda and Swarup (1966,1967), Shome and Shome (1966), Sachchidananda and Ram Krishnan (1971) and Patil and Pendse (1976, 1981). Many nematode-trapping fungus species have been reported by various researchers as new records from India (Dayal and Nand, 1973a, 1973b; Dayal andSingh, 1975; Dayal and Gupta, 1975b; Dayal and Srivastava 1978; Srivastava, 1981, Srivastava and Dayal, 1982, 1984; Prasad *et al*., 1984; Prasad, 1985). The taxonomy of the fungi has been a major problem for scientists conducting research on nematode-

trapping fungi. Drechsler listed a number of nematode-trapping fungal species that capture, kill, and digest the nematodes. Soprunov (1951), Subramaniam (1963), Cooke and Godfrey (1964), Cooke and Dickinson (1965), Rifai and Cooke (1966), Cooke (1969), and others have focused their studies on the taxonomy of nematode-trappingfungi. The species of nematode-trapping fungus have commonly been identified using a key of nematode-trapping fungi provided by Cooke and Godfrey (1964). Reference material for studies on nematophagous fungus may be found in the book The nematode destroying fungi written by Barron (1977).

2.2 Molecular characterization of nematode trapping fungi

Pandit RJ et.al., (2014). isolated two different nematode trapping fungi from arable soil samples and identified based on their cultural morphological and 18S rRNA gene sequencing as *Arthrobotrys conoides* (Genbank accession No. jx979095) and *Duddingtonia flagrans* (Genbank accession No. JX979094) and assessed for their trapping efficiency against root knot disease (*Meloidogyne* spp.)

Chauhan JB et al., (2006). isolated *Arthrobotrys musiformis* which was found to against *Haemonchus contortus* that affects milk and meat production in ruminant. He also showed that B tubulin gene of *A. musiformis* was isolated from genomic DNA through PCR and sequenced BLAST analysis revealed 95% homology with β-tubulin gene of *A. oligospora* followed by other filamentous fungi.

Falbo MK. et al., (2012). isolated two pure isolates namely CEN & LIN and show 96.35 % carnivorous activity for strain CED and was selected and characterized by using molecular methods by sequencing the rDNA ITS and was identified as *Arthrobotrysconoides* (Gen-bank ID: JN191309

Wang, FH. et al., (2017). looked for native isolates of *A. Thaumansia* and characterized them using light microscopy and molecular markers and reveal the effect of pH, nutrition and temperature on growth and trap formation. They sequemnce the nrDNA internal transcribe spacer of isolate NBS005 by using BLAST and submitted to Gen-bank (ID: KX640093).

Ocampo-Gutiérrez, A. Y. et al., (2021). isolated and molecularly characterized 4 nematophagous fungi *viz*. *A. conoides, A. oligospora, A. arthrobotryoides* and

Purpureocillium lilacinum and show the carnivorous efficacy against *Haemonchus contortus* and *Panagrellus redivivus.*

2.3 Research on trapping structure of nematode trapping fungi

Predatory fungus produces trapping structures in response to nematodes or additional environmental factors. Predacious fungi have been known to spontaneously form traps for a long time, but little is known about the conditions that lead to this spontaneous trap development. Many researchers have been interested in studying this topic since the process of trap formation in fungi that are predators has been an intriguing phenomenon.

Couch (1937), found that trapping rings of were formed infrequently or not at all, in pure culture of this fungus, studies on trap generation by predatory fungi in thepresence of nematodes. However, when some water containing nematodes was put to a pure culture of *Dactylaria bembicodes,* many rings began to develop. They discoveredthat adding sterile nematode culture filtrates to a pure culture of the predatory fungus might cause the traps to develop. His finding makes it very evident that nematodesproduced substances that led to the development of trapping mechanisms. Later research by Roubaud and Deschiens (1939) demonstrated that the trap-inducing substance was not exclusive to nematodes. Among these, earthworm extracts and human blood serum were significantly more efficient.

The word "nemin" was proposed by Pramer and Stoll in 1959 to describe the compounds that cause trap formation. They established that *Neoaplactana glaseri*, a nematode, produces this chemical compound as a trigger for the development of traps in predatory fungus. Additionally, they noticed that nemin production did not occur during periods of fast growth and multiplication but rather began when nematodes were fully developed. It was demonstrated through several dilutions of nematode culture filtrates that an ideal concentration of nemin alone could create a significant number of traps. The creation of an abundance of traps was best at 1/5 the strength of the culture filtrate. Even though nemin's solubility characteristics with various compounds were sufficiently elucidated, its composition was not identified. They claimed that 10 minutes of exposure to 100^0C did not render nemin inactive.

Tarjan (1960) stated that *Penagrellus redivivus* is known to generate traps in fungi that feed on other organisms. He noticed that the number of nematodes determined by how many traps are formed.

Feder et al. (1960) noted that a single dried nematode caused trap formation over a *Dactylella doedycoides*-containing 1 cm column. This made it very evident that very little

nemin was necessary for the induction of traps.

Pramer and Kuyama (1963), stated that nemin was either a low-molecular-weight peptide or potentially just a single often occurring amino acid. Although they tested 13 proteins, 49 peptides, and 27 amino acids, they were unable to cause morphogenic alterations in fungus that were predatory.

Feder et al. (1963) used a higher dilution of nematode population than had previously been used by Pramer and Stoll (1959) to observe trap formation in predatory fungi. Additionally, they proposed that the majority of the nemin was present as endogenous nemin in nematode bodies. They carefully examined how endogenous nemin from *P. redivivus* affected the morphogenesis of five species of *Dactylella* and noticed variations in how these fungi responded to nemin. Even at a 10^{-6} dilution, *Dactylella cinopaga* showed signs of morphogenesis, whereas *D. bembicodes* and *Dactylella drechsleri* were unresponsive to 10^{2} and 10^{-3} dilutions, respectively. When it came to *Dactylella ellipsospora*, they discovered that while it did not respond to endogenous nemin, it exhibits morphogenesis when grown in culture with live nematodes. This explains the difference in chemical makeup between endogenous nemin and the extract of live nematodes.

Bartniéki-Garcia et al. (1964), reported that trap formation in two strains of *A. conoides* in the presence of nemin was CO_2 dependent. Without CO_2, no traps developed. The highest CO_2 concentration (10%) formed the greatest number of traps in one of the fungal strains, even though the ideal CO_2 concentration was not identified.

Nordbring-Hertz (1973) established that amino acids stimulate trap formation. However, the impact of peptides, particularly valyl peptide, was substantially stronger. Based on her findings, she concluded that various peptides might induce traps in *A. oligospora*, *A. superba,* and *Monacrosporium eudermatum*. She also discovered that the degree of nutrients of a medium was crucial in trap induction. She went on to say that the peptides capable of causing morphogenesis were created by the biological breakdown of proteinaceous substances in the soil.

Monoson et al. (1974) observed that endogenous nemin isolated from five distinct species of nematodes caused trap formation. When combined with different nemins, the RNA synthesis inhibitor 6-methyl purine fully blocked the fungal trap formation. One hour after the addition of different nemins, the application of 6-methyl purine had no influence

on the number of traps formed. Two drugs that disrupt normal protein synthesis (5-thiouracil and Pfluorophenylalanine) also caused trap formation in *Monacrosporium doedycoides*, which appeared to be at the transcriptional level.

Predacious fungal trapping structures have been found to be caused by the means other than nematodes or animals. Soprunov (1958) discovered that adding mycelial piecesto melting snow at ambient temperature or rainfall generated traps. When spores ofpredacious fungus germinated in rainwater containing 1-2% ethyl alcohol, he discovered the most frequent and fast development of traps. He discovered that when $C0_2$ in rainfall was combined with ammonium compounds, ammonium carbonate was produced, which caused trap formation. He demonstrated that morphogenic activity was diminished owing to nematode filtrate sterilisation.

Ring formation is also known to be triggered by nutritional insufficiency and water stress. In poor conditions (lack of nutrients or water), *A. dactyloides* and *D. brochopaga* developed many traps that remained functional and capable of collecting nematodes, according to Balan and Lechevalier (1972). They compared the number of traps formation in the presence of nematodes to the number of traps formed in the presence of fungal culture on cellophane paper. The number of rings formed in double layer cellophane papers was comparable to the number of rings formed in the presence of nematodes *P. redivivus*.

Lawton (1957) noticed that morphogenic substances were not necessarily required, and there was evidence of spontaneous trap development on the hyphae of predacious fungus in culture. He discovered that the spontaneous trap development in *A. dactyloides* and *D. brochopaga* was accelerated by contact with glass and was not caused by fungal starvation.

Bartnicki et al. (1964) discovered that trap formation in two strains of *A. conoides* was CO_2 dependent in the presence of nemin. In the absence of CO_2, no traps developed. Though the optimal concentration of CO_2 was not found, the highest concentration (10%) produced the greatest number of traps in one of the fungal strains

2.4 Research on nematophagous behaviour of nematode-trapping fungi

Cooke and Pramer (1968), reported that the presence of the mycophagous nematode, *A. avenae* had no effect on the rate of colony formation of nematode-trapping fungus. They found that the nematophagous activity of these fungus had no effect on the nematode

population. Nematode-trapping ability of fungi initially reduced nematode population, but later on predation became low owing to poisonous compounds generated by nematodes, which finally contributed to the demise of these fungi. As a result, after 30 days in dual culture with *A. avenae*, nematode-trapping fungi were not obvious.

Monoson (1968) investigated the influence of temperature on the development and trapping efficacy of several nematode-trapping fungus. The majority of his researchfocused on the growth of *A. oligospora, A. musiformis, A. dactyloides, Monacrosporium bembicodes,* and *M. cionopagum* in connection to temperature and the population of *A. avenae* and *Neotylenchus linfordi*. He noticed that there was minimal variation between enhanced trapping structures and temperature. Furthermore, the presence of too many nematodes did not promote the growth of nematode-trapping fungus. His discovery also confirmed Cooke and Pramer's (1968) theory that an increased population of nematodes caused less growth, resulting in a negative association. The best predation of *A. oligospora, A. musiformis,* and *M. bembicodes* against *A. avenae* was found at 15-200C, but *M. cionopagum* was most effective at 200C on V-8 agar medium and *A. dactyloides* at150C on PDA, 250C on CMA, and 300C on V-8 agar media. However, independent ofthe medium or the number of nematodes present in the inoculated sample, all of the nematode-trapping fungus captured *N. linfordi* very successfully at all temperatures. In plates containing 100 nematodes, a high percentage of nematode-trapping was observed.

Heintz (1978) found that the age of the fungus colony, the number and kind of nematodes supplied, and the fungus' capacity to build traps within the agar influenced nematode trapping and parasitism. He also computed the predacity index (PI) undervarious experimental situations. The greater the PI value, the greater the capability for predation. Esser et al. (1991) investigated the interaction of *Dactylella megalospora* with 18 species of Phyto nematodes and 9 free-living nematodes and found that all nematodes were captured and absorbed by the fungus, while certain nematodes were resistant to entrapment.

Belder and Jansen (1994) investigated the capacity of *A. oligospora, M. cionopagum, A. dactyloides, A. scaphoides, and Duddingtonia flagrans* to parasitize *Meloidogyne hapla, M. incognita, Globodera pallida,* and *G. rostochinensis in vitro*. They discovered that *A. oligospora* and *M. cionopagum* isolates were particularly successful in capturing *M. hapla* and *M. incognita*. The capturing efficiency of *Arthrobotrys conoides, A. dactyloides,* and *A.*

scaphoides was moderate. However, regardless of the age of the mycelium, capture by some isolates of *Arthrobotrys* and *Duddingtonia* did not occur. Furthermore, they discovered that while the isolates were highly efficient against *M. hapla* and *M. incognita*, they had limited capacity to trap other nematodes such as *G. rostochinensis* and *G. pallida*. They determined that nematode- trapping fungus is unique in their capacity to capture nematodes.

Belder and Jansen (1994a) investigated the influence of temperature, substrates, light, and mycelia ageing on the capacity of an isolate of *A. oligospora* (CBS - 29882) to catch *M. hapla (J2) in vitro.* They found that trapping of nematodes by the fungal mycelia took less than an hour, regardless of temperature or worm mobility. However, trap growth was substantially lower at lower temperatures (5-10^0 degrees Celsius) than at higher temperatures (15-30^o degrees Celsius). They also found that nutritional circumstances had no effect on nematode hyphae adhesion, but that the establishment of an adhesive network was delayed on water agar. The capturing efficacy of fungal mycelia did not alter with age up to 70 days. Light had no effect on nematode capture as well.

de Gives et al. (1994) assessed the nematophagous capacity of *A. oligospora* and *A. conoides* against the third stage and second stage larvae of *Haemonchus contortus* and *Nacobbus aberrans*, *in vitro* at various temperatures respectively. At 18°C and 25°C, respectively, *Arthrobotrys oligospora* exhibited 35.87% and 25.71% trapping. No capture was noticed since none of the fungi were identified to form three-dimensional sticky loops at 30^0C. For both nematodes, *A. conoides* had a capture efficiency of more than 90%.

Cayrol and Sawadogo (1991) noted that the *M. bembicodes*' three-celled constricting ring had captured the nematode within a week of inoculation. They also discovered that the presence of fungus caused 80% hatching inhibition.

Galper et al. (1995) created straightforward screening techniques to evaluate the nematophagous capacity of nematode-trapping fungi. They discovered that these fungi form traps in response to *Caenorhabditis elegans*. Additionally, they observed that the juvenile *M. javanica* population induced fewer traps and took longer than three days to reduce more than 90% of the nematodes, in contrast to the *C. elegans* population, which was reduced by at least 90% within three days. *A. dactyloides* and *Dactylella candida* consistently reduced the number of *M. javanica* juveniles recovered from the soil, whereas *Monacrosporium sp., A. oligospora, A. conoides,* and *A. musiformis* did not produce the same results. In order to

track trapping activity in soil, they also developedthe agar "sandwich" and buried slide techniques. They came to the conclusion that *D. candida* and *A. dactyloides* consistently generated the traps within 5 days after introduction, whereas network-forming species created less traps in the soil.

Cooke (1977), investigated that nematode-trapping fungus can only collect and kill nematodes for a brief amount of time following the start of soil amendment degradation. He noticed that, despite an increase in nematode population, increasing the modifications over a certain threshold led to a decrease in parasitic activity. He came to the conclusion that nematodes weren't the only source of nutrition for nematode-trapping fungus, but rather one of several. The nematophagous habit was allegedly developed to lessen oravoid competition for accessible substrate during the breakdown of organic matter in soil, and nematode-trapping fungi were only active during times of competition, according to his theory.

2.5 Biological control of plant parasitic nematode by nematode-trapping fungi

Numerous researchers have addressed the biological control of plant parasitic nematodes by nematode-trapping fungus (Duddington, 1962; Mankau, 1980; Siddiqi and Mahmood, 1997). The number of free-living nematodes in the soil had significantlygrown, as reported by Linford (1937) and Linford et al. (1938), and they came to the conclusion that this had fostered the growth of nematode-trapping fungus, which in turn had killed off the nematodes, returning their population down to its original level.

Out of five species of nematode-trapping fungus deployed in the soil for the biological control of plant parasitic nematodes, Linford and Yap (1938, 1939) found that only a handful offered minimal control of nematodes. However, nematode-trapping fungus demonstrated improved bio-control when they were added to soil containingorganic matter. For the biological control of *M. incognita* on tomatoes, Ali (1990) evaluated the nematophagous efficacy of *A. oligospora* and organic amendments. He found that when *A. oligospora* was inoculated two weeks before transplanting and nematode inoculation, it gave a 72% reduction in the number of root knots. Slepetiene et al. (1993) *A. oligospora* was shown to be more successful (46.581.9%) than the use of chemical against root-knot induced by *Meloidogyne sp.*

Colombo et al. (1995) tested a commercial formulation of the nematode-trapping fungi *A. oligospora* and *A. superba* against the brinjal root-knot disease, they found that while the formulation was successful in reducing nematodes in the soil, it was lesssuccessful in reducing the number of root knots when compared to treatment with Fenamiphos. According to Arndt (1994), *M. incognita* population in tomato plant was decreased by *A. oligospora* and *A. dactyloides.*

Stirling et al. (1998), found that *A. dactyloides* formulations reliably reduced the population of *M. javanica* juveniles by more than 90%. They also noted a 57-96%decrease in the number of galls caused by the root-knot nematode in field soils treated with granules (10g/litre) and planted with tomatoes. According to Stirling and Smith (1998), the formulations of *A. dactyloides* treated at 220-440 kg/ha significantly decreasedthe number of nematodes present in roots 4–8 weeks after planting but the formulations of *V. chlamydosporium* did not significantly reduce galling.

Kumar and Singh (2006) found that mass-culturing *A. dactyloides* in soil contaminated with 2000 juvenile *M. incognita* per "kg" of soil prior to planting of tomato seedling reduced the number of root knots by 5.6-45.6%, females by 44.7-72.9%, egg masses by 44.5-51.3%, and juveniles by 37.9-81.8%, while increasing plant growth.When this fungal mass culture was combined with cow dung manure, it had a greater impact as a biocontrol agent, reducing the quantity of root knots by 61.7–66.6%, females by 80.6–94.7%, egg masses by 80.3–89.6%, and juveniles by 68.1–88.0%.

Singh et al. (2007), found that the introduction of *A. dactyloides* and *D. brochopaga* into *Meloidogyne graminicola*-infested soil, respectively, resulted in an 86% and 94% decrease in the amount of root galls, females, eggs, and juveniles. When compared to these parameters for plants grown in soil with *Meloidogyne graminicola* infestation, the biocontrol potential of these fungi increased plant growth by 42.7% and 39.8% in shoot length, 45.5% and 48.9% in root length, 59.9% and 56.7% in fresh weight of shoot, and 20.3% and 25.1% in fresh weight of root, respectively.

Kumar and Singh (2011), found that, applying *D. brochopaga* mass culture and spore suspension to soil contaminated with *M. graminicola* juveniles both with andwithout cow dung manure significantly decreased the number of root-knots, egg masses, juveniles, and females in comparison to the control. When cow dung manure was combined with a mass

culture and spore suspension, the fungus' bio-efficacy was increased.

Simon (2011), investigated that the administration of mass cultures of *Arthrobotrys oligospora* and *Dactylaria eudermata* decreased the number of females, rootgalls by 94.2% and 91.7% and the number of root galls by 86.9% and 81.1%, respectivelyOver nematode-infested soil. The mass culture of such fungi accelerated plantdevelopment, increasing shoot and root lengths by 41.9%, 38.8%, 61.1%, and 58.7%, respectively, as well as the fresh weight of the shoot and root.

Singh et al. (2012a), found that under greenhouse and mini plot (field) conditions, the treatment of *A. oligospora* in soil infected with *M. graminicola* and *R. solani* decreased the number of root knots by 57.58-62.02%, sheath blight incidence by 55.68- 59.32%, and lesion length by 54.91-66.66%.

Singh *et al*. (2012b) found that the co-inoculation of *D. brochopaga* Dp-5 and *M. eudermatum* Mv-1 significantly reduced root-knot disease in tomato (89.63%) and increased the accumulation of total chlorophyll (125.34, 140.53 and 152.67 mg g^{-1} fresh wt.), total phenolic compounds (TPC) (37.40, 48.32, and 59.63 µg of gallic acid equivalent), and phenylalanine ammonia lyase (PAL) activity (58.45, 69.05, and 74.57mm cinnamic acid h^{-1} g^{-1} fresh wt.) after 10, 20 and 30 days of inoculation, respectively, in the greenhouse.

Singh et al. (2012c), found that the plants treated with *Arthrobotrys oligospora* exhibited improved growth in terms of shoot and root length and biomass, chlorophyll and total phenolic content, and high phenylalanine ammonia lyase activity when compared to plants infected with *M. incognita* and *R. solani*. They claimed that *A. oligospora* has the capacity to offer bio-protection agents against *R. solani* and *M. incognita*. Additionally, they proposed that using *A. oligospora* will improve tomato fruit nutrition and plant development in addition to aiding in the control of nematodes. This makes it both a great biocontrol agent and a growth-promoting agent for plants.

Singh et al. (2013) investigated the biocontrol potential of the nematode-trapping fungus *Dactylaria brochopaga* against *Anguina tritici* and *Meloidogyne graminicola* and found that co-inoculation of *D. brochopaga* and *C. anguillulae* significantly reduced root-knot and seed gall in wheat and increased plant growth parameters compared to pathogen challenged plants without any bioagents/chemical nematicide. Singh et al. (2014)

investigated the ability of *Catenaria anguillulae* and *Dactylaria brochopaga* to colonise wheat seed gall and diminish *M. graminicola* and *A. tritici* in wheat (*Triticum aestivum* L.). Under greenhouse conditions, co-inoculation of *D. brochopaga* and *C. anguillulae* significantly reduced root-knot and seed gall in wheat and increased plant growth parameters such as length and dry weight of root and shoot as well as yield attributing characters such as spike length, number of seed per spike, test weight, and so on as compared to pathogen challenged plants that did not receive any bioagents/chemicalnematicide. Singh et al. (2019) studied the effect of *Drechslerella dactyloides* and *Dactylaria brochopaga* in root apoplast reprogramming to increase defensive responses intomato pre-challenged with Meloidogyne incognita. *D. dactyloides* and *D. brochopaga* were determined to be of the greatest potential strains for *M. incognita* management.

Singh et al. (2020) observed the diverse impacts of *Dactyloides* and *D. brochopaga* when infected separately or in combination in tomato plants that had previously been challenged with *M. incognita*. Furthermore, *D. dactyloides* and *D. brochopaga* greatly improved antioxidant and biocontrol activities in tomato against *M. incognita*. Microscopic examination of H_2O_2 and superoxide radicals in tomato leaves supported the previous findings. Furthermore, inoculation of *D. dactyloides* and *D. brochopaga* triggered the phenylpropanoid pathway in roots, resulting in increased cell wall lignification and pectin deposition in tomato roots, as well as direct trapping and parasitizing of *M. incognita* juveniles and adults. As per the findings, enhanced cell wall lignification and pectin deposition likely prevented nematode entrance and, as a result, reduced the *M. incognita* population in tomato roots. Plants treated with nematode- trapping fungus, either separately or in combination, altered phenotypic changes andaided plant growth promotion.

2.6 Nematode-trapping fungi and their mycoparasitic behavior on *R. solani*

Nematode-trapping fungi are a class of fungi that parasitize the nematodes and hyphae of the fungus *Rhizoctonia solani* in various ways. These fungi parasitize nematodes by capturing and killing them with particular hyphal traps, and they parasitize

R. solani by producing hyphal coils around its hyphae. As a result, these fungi become increasingly crucial in regulating and sustaining the population of nematodes and *R. solani* in soil. After Fresenius discovered *Arthrobotrys oligospora* in 1852, knowledge about these

fungi began to accumulate.

Woronin (1870) observed that when *A. oligospora* spores germinated on old manure, part of the hyphae formed hyphal nets, but he did not know what these nets were for.

Zopf (1888) discovered that these hyphal nets trap moving nematodes. He discovered fungal hyphal development within the nematode body and penetration of the caught nematode's cuticle. He was the first to discover the biological connection between a predatory fungus and a nematode. His descriptions of the process of predatism manifestation emerged in *'Nova acta Leopoldina Carolina Academia,'* which isconsidered one of the finest masterpieces in mycological study.

Drechsler (1933, 1933a) revealed that nematode-trapping fungus produced potent adhesives that were responsible for nematode capture. Initially, Drechsler focused on *Pythium* and *Phytophthora*. During his investigations, he discovered that certain fungus attacked nematodes in plates that were essentially plated for the isolation of *Pythium* and *Phytophthora* on a low nutrition agar medium. Drechsler's perspective on nematode-trapping fungus was fundamentally altered by the dynamic interplay between fungi and nematodes. Drechsler was inspired by the intriguing trapping methods of nematode-catching fungus and dedicated his life to studying them.

Duddington (1952-72) was a pioneer in the study of nematode-trapping fungus. He came to the conclusion that nematode-trapping fungi are abundant in natural soils, agricultural soils, and all types of decomposing manures. He also explained how to handle nematode-trapping fungus (Duddington 1955). His book "The Friendly Fungi" (Duddington 1957) discusses many elements of nematode-destroying fungus. Severalother researchers from across the world have also contributed to this significant group of fungus via their research (Juniper 1957; Pramer and Stoll, 1959; Cooke, 1962, 1962b, 1963; Higgins and Pramer, 1967; Barron, 1969, 1977; Mankau and Clark, 1959; Monosonand Ranieri, 1972; Pramer, 1964; Nordbring-Hertz *et al.,* 1977; Stirling *et al.,* 1998a; Jansson and Nordbring–Hertz, 1980; Gray, 1983a, 1983 b and Stirling *et al.,* 1998b).

The Indian employees paid little attention to this fascinating variety of fungi. Das Gupta et al. (1964), Sachchidananda (1965), Sachchidananda and Swarup (1966, 1967), Shome and Shome (1966), Sachchidananda and Ram Krishnan (1971), and Patil and Pendse

(1976, 1981) all began basic research on nematode-trapping fungus. Detailed experiments on nematode-trapping fungus were conducted in India from 1973 to 1985 under the direction of Prof. Dayal and his students. Despite the fact that their research on nematode-trapping fungus was limited to saprophytic nematodes, their contribution was significant. They have documented a vast number of nematode-destroying fungus, manyof which are new records from India (Dayal and Nand, 1973a, 1973b; Dayal and Singh, 1975; Dayal and Gupta, 1975b; Dayal and Srivastava 1978; Srivastava, 1981, Srivastava and Dayal, 1982, 1984; Prasad *et al.*, 1984a; Prasad *et al.*, 1984b; Prasad, 1985).

Butler proposed the terms mycoparasitism and mycoparasite to describe one fungus to parasitize on another. Persson et al. (1985) studied the mycoparasitic behaviour of nematode-trapping fungi by constructing a hyphal coil around the fungal hyphae. A thorough investigation using *Arthrobotrys oligospora* demonstrated that diverse fungus from all taxonomic groups were attacked. In dual cultures, the interaction between *A. oligospora* and *Rhizoctonia solani* occurred shortly after hyphal contact, regardless of medium nutritional content. When the two organisms were cultivated on sterilised soil, coiling was also seen. Fluorescence microscopy using fluorescein diacetate (FDA)-stainedsamples revealed that the coils had a high metabolic activity relative to the surrounding hyphae. Ultrastructural examination of emerging coils revealed a large number of membranous vesicles derived from tubular-shaped endoplasmic reticulum. Strong cell wall proliferation was found in *Rhizoctonia* cells at the coiling location. These cells' cytoplasm subsequently dissolved. Vital staining studies proved the cell's demise. There was no penetration of complete *Rhizoctonia* cells. The relationship between *A. oligospora* and *R. solani* is viewed as nutrient competition.

Persson and Bååth., (1992) used coiling frequency to investigate the effect of nutritional content, carbon source type, and nitrogen concentration on *Arthrobotrys oligospora* parasitism on *Rhizoctonia solani*. The frequency of coiling was also compared to the density of hyphae and the pace of colony radial expansion. Corn meal agar concentrations were increased to half the optimum strength to increase coiling frequency. The coiling frequency remained constant at increasing doses, despite the fact that the hyphal density of both fungi increased over the whole concentration range. Except at high CMA concentrations, coiling frequency was positively linked with the chance of hyphal meeting, defined as the product of the hyphal densities of the two fungi. Among the carbohydrates

studied, glucose had the highest coiling frequency and sucrose had the lowest. The influence of various carbohydrates on coiling frequency was not connected to the fungi's hyphal densities. The addition of a nitrogen supply, $NaNO_3$, eliminated the coiling frequency disparities between glucose and sucrose and raised coiling frequencies on both sugars.

Singh et al. (2012) investigated the mycoparasitic activity of five *Arthrobotrys oligospora* isolates against *Rhizoctonia solani in vitro*. They discovered the formation of and hyphal coils around *R. solani* hyphae. For the first time, they proved the bio-control capacity of *A. oligospora* against *R. solani*, which causes sheath blight in rice (*Oryza sativa* L.). Under green house and small plot (field) conditions, the application of *A.oligospora* isolate VNS-1 to soil-infested *R. solani* reduced sheath blight incidence by 55.68-59.32% and lesion length by 54.91-66.66%.

3.1 Glassware cleaning and sterilization

Glass wares from Corning and Borosil were used for all laboratory experiments. The glassware was first boiled for 30 minutes, followed by a vim powder wash, washingin tap water, and rinsing with distilled water. Petri dishes, culture tubes, funnels, beakers, flasks, watch glass, and glass rods were among the glass items cleaned with cromic acid (60g of potassium dichromate, 60ml of sulphuric acid concentration, and 1000ml ofwater). Prior to use, Petri dishes were sterilized in a hot air oven for two hours at 160°C.

3.2 Media preparation

3.2.1 Preparation of Potato Dextrose Agar-

PDA medium was utilised in the majority of the experimental experiments. Potato dextrose agar (PDA) medium was utilised for in vitro investigations for the isolation and purification of fungal cultures. The following was reported regarding PDA's composition:

Peeled potato	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml (Volume to make up)

200 grammes of peeled potato were chopped into little pieces and cooked in distilled water, with the extract collected by filtering through muslin fabric. Dextrose (20 g) and Agar-Agar powder (20 g) were dissolved in potato extract, and the final volume was filled with distilled water to 1000 ml. It was then sterilised at 121.6^0C temperature, 1.1 kg/cm^2 pressure for 15 minutes and stored for future use.

3.2.2 Preparation of Corn Meal Agar: -

Corn meal agar (CMA) medium was employed for nematode-trapping fungal isolation and purification, as well as in vitro investigations. The following isthe composition of CMA:

Broken corn grains	:	20g
Agar agar	:	20g
Distilled water	:	1000 ml (Volume to make up)

After boiling 20 grammes of broken maize grains in distilled water, the extract was obtained by filtering through muslin fabric. Agar agar powder (20g) was dissolved in maize extract, and the final amount was filled with distilled water up to 1000ml. It was then sterilised at 121.6^{0}C temperature, 1.1 kg/cm2 pressure for 15 minutes and stored for future use.

3.2.3 Preparation of Rabbit Dung Agar: -

Rabbit dung agar (RDA) medium was employed for growing and sustaining nematode populations as well as in vitro investigations. The following is the composition of RDA:

Rabbit dung	:	100g
Agar agar	:	20g
Distilled water	:	1000 ml (Volume to make up)

100 grammes of rabbit dung was steeped in distilled water for 24 hours before being filtered through muslin fabric. Agar agar powder (20 g) was dissolved in hot rabbit dung extract for full agar agar dissolution, and the final volume was brought up to 1000ml with distilled water. It was then sterilized at 121.6^{0}C temperature, 1.1 kg/cm2 pressure for 15 minutes and stored for future use.

3.3 Symptoms collection and isolation of *R. solani*

Rice plant showing the typical symptoms of sheath blight lesions and web blight appearance on the leaf of mung bean were collected from agriculture Research farm of Banda University of Agriculture & Technology and fungus was isolated by standard tissue isolation method. Both the samples were washed in the running tap water, and then it was cut into 1-2 cm bits. Bits were surface sterilized in the 0.1 per cent mercuric chloride for 30 seconds then bits were washed twice in the sterilized distilled water under aseptic condition. Bits were again cut into 0.2 cm and then these bits were transferred to PDA plates aseptically, incubated at 27±2^{o}C for ten days. Based on morphological and cultural characteristics isolates were identified as *R. solani* AG-1 of rice and *R. solani* AG-3 of Mung bean. The

culture of isolates of *R. solani* were sub cultured periodically at an interval of 10 to 15 days and were mentioned on PDA medium at 25 ±1°C.

3.4 Isolation of different nematode trapping fungi

The soil sprinkling technique first reported by Drechsler (1941) and modified by Duddington (1955) with minor changes incorporated by (Singh et al., 2007, Kumar et al., 2005) was used to isolate several nematode-trapping fungi. 500 g of soil samples were collected in separate plastic bags from the top profile of soil in several Bundelkhand locations. Sterilized corn meal agar medium (broken corn grains: 20 g, agar-agar: 20 g, and distilled water: 1000 ml) was placed into numerous sterile Petri dishes to fill roughly two-thirds area of a culture plate. After the corn meal agar medium had solidified, melted and cooled rabbit dung agar (rabbit dung pellets-100 g, agar-20g and distilled water-1000 ml) media was put into these Petri plates to fill the remaining space. Each soil sample waswell mixed, sieved (2mm pore size), and about one gramme was sprinkled over the poured medium onto Petri plates. Five Petri plates were utilised as duplicates for each soilsample. Room temperature (25-30°C) was used to incubate the Petri plates. Under a binocular compound microscope, incubated Petri dishes were studied daily after one weekfor the incidence of trap formation, capture of nematodes, and production of conidialheads of nematode-trapping fungi. A sterilised fine needle was used to pick up the spores of nematode-trapping fungus formed on single conidial heads close to the captured nematodes under a compound microscope and transfer them individually into the corn meal agar medium for the isolation of individual nematode-trapping fungal species. For growth and sporulation, the spores inoculated into Petri dishes were incubated at 25°C. After 10 days, spores from each fungal species were put back into Petri plates containing corn meal agar media. Single spore isolation was used to further purify distinct kinds of nematode-trapping fungus. Pure cultures of each nematode-trapping fungus species were grown on corn meal agar medium at 25°C.

3.5 Identification and characterization of different nematode trapping fungi

A calibrated ocular micrometer was used to assess the size of conidia, conidiophore, hyphae, and trapping structures at different magnifications (10x, 20x, 40x and 100x). A bright-field microscope, an ocular micrometre scale, a stage micrometer, and oil immersion were utilized to calibrate the ocular micrometer. The glass disc of an ocular micrometer was

placed on the metal diaphragm, and the eyepiece was placed in themicroscope. The number of ocular and stage micrometer divisions between the twointersecting lines was counted. Five readings were collected with the high-power (10x, 20x, 40x), oil immersion (100x) objectives. These observations are then utilized to compute the calibration factor for the current objective lens.

$$\text{one ocular division } (\mu m) = \frac{\text{No.of division on stage micrometer}}{\text{No.of division on ocular micrometer}} \text{ X } 100$$

After calibration, the ocular micrometer was used to calculate the length, breadth, and diameter of hyphae, conidiophores, conidia etc. by using following formula.

Size of microorganism (in micron) =Number of ocular micrometer divisions occupied x

Calibration factor for one ocular division

(For the objective lens used)

The size as well as the shape of every morphological structure of the nematode-trapping fungi were measured, and the results were compared to original descriptions given by Drechsler (1937), Drechsler (1950), Drechsler (1952), Drechsler (1954), and Cook and Godfrey (1964) for the purpose of identification of all the nematode-trapping fungi isolated during course of present investigation.

3.6 Molecular characterization of nematode trapping fungi

3.6.1 Multiplication of different isolates and preparation of hyphal mat

Various isolates of nematode trapping fungi were grown on cornmeal agar media (CMA) by single spore isolation method and sub cultured periodically at an interval of 10 to 15 days and were mentioned on CMA medium at 25 ±1^{o}C. Preparation of hyphal mat was carried out on potato dextrose broth (PDB) medium. A 5mm hyphal disc were cut with help of sterile cork borer from freshly grown pure culture under aseptic condition and transferred to the sterilised PDB then incubated in a shaking incubator at 25 ±1^{o}C.The hyphal mat was ready to harvest in about 10-15 days (depends on growth of isolate).

3.6.2 Harvesting and disruption of mycelial mat

After 10-15 days of incubation, the mycelial mat was harvested by using Whiteman No. 1 filter paper, slightly rinse with sterilized distilled water to remove the media particle and dried the mycelia well by using blotter paper. All the work were carried out in aseptic condition.

3.6.3 Isolation of fungal DNA

3.6.3.1 List of chemicals used

Chemicals employed in current investigation are given in appendix A.

3.6.3.2 Preparation of stocks and buffer solutions

Protocols for the preparations of stocks and buffer solutions used in this study are written in appendix B.

3.6.3.3 List of equipment used

Equipment that are used in this study are given in the appendix C.

3.6.4 Steps in DNA isolation

- Crush the 100mg of fresh mycelia to powder form using liquid nitrogen add 750 µl 3 % CTAB buffer while griding.
- Transfer in 2 ml centrifuge tube and add 2 µl β merceptoethanol then vertex the sample for 2-3 min. with vertexor
- Put it at 65^{o}C in water bath for 1 hr with slight hand vertexing in every 15 min.
- After 1 hr put the tube at room temp. for 10 min
- Then sample was centrifuged at 12000 rpm for 15 min at room temp.(28^{o}C)
- Transfer the 500 µl supernatant in 1.5 ml centrifuge tube and equal amount of CI (24:1 Chloroform: isoamyl alcohol) and leave it for 15 min after slight vertexing.
- Now centrifuge the sample at 12000 rpm for 5 min at room temp.
- Again, Transfer the 500 µl supernatant in 1.5 ml centrifuge tube and equal amount of CI (24:1 Chloroform: isoamyl alcohol) and leave it for 15 min after slight vertexing.
- Now centrifuge the sample at 12000 rpm for 5 min at room temp.
- Transfer the 200/400 µl clean supernatant in 1.5 ml centrifuge tube and add 1.5 times (300/600 µl) chilled isoprapanol and keep it at -20^{o}C for 30 min.
- Then centrifuge at 9000 rpm for 15 min. at 4^{o}C
- Discard the supernatant and keep the tube open to evaporate the alcohol until the smell was lost (overnight)
- Add 50 µl nuclease free water and store the final DNA at -20^{o}C

3.6.5 Assessment of quality and quantity of DNA

DNA was assessed for its purity and quantity using both agarose gel and Nano

Drop spectrophotometer.

3.6.5.1 Quantification of DNA by 0.8% agarose gel electrophoresis

Preparation of 1% agarose gel:

In a conical flask holding 100 ml of 1X TBE buffer, 1g of agarose was added. The agarose gel was put in the conical flask with its contents and heated in the oven until a clear solution appeared. Once the solution had cooled to between 50 and 55^0C, the flask was removed from the oven. 100 ml of agarose gel was combined with 2 µl of ethidium bromide (10 mg-1) and thoroughly mixed. Later, this solution was poured into the gel casting tray gently, to prevent bubbles formation which was pre-set with 0.5 mm combs. After the gel had solidified, the comb was carefully removed, and the gel with the casting tray was then put in the gel tank.

3.6.5.2 Electrophoresis of the DNA samples:

4 µl of dissolved genomic DNA samples were combined with 2 µl of 6X Gel loading dye (40% sucrose and 0.25% bromophenol blue) and loaded onto a 1% agarose-1XTAE gel. The gel containing loaded samples was then electrophoresed for roughly an hour at 80V at room temperature. The gel was then visualized in a UV gel documentationsystem and the image was preserved for future use. The intensity and thickness of genomicDNA was then compared to that of DNA, and the concentration of DNA in each sample was calculated.

3.6.5.3 Quantification of DNA by Nano Drop method:

The Nano drop spectrophotometer method was used to test the quantity and quality of DNA using the protocol outlined below.

- The pedestal was cleaned with tissue paper before starting the Nano drop Reader toeliminate dust particles.
- After that, 2 µl of distilled water was put on the pedestal and the measure option wasselected.
- The pedestal was then cleared with tissue paper, and 2 µl of 1X TE buffer was put onit for blank measurement.
- Finally, the pedestal was wiped with tissue paper before placing 2 µl of DNA sample to determine the quantity and purity of DNA.

The absorbance ratio at 260 nm and 280 nm was used to determine the purity of DNA. A ratio of 1.5 is widely regarded as "pure" for DNA. If the ratio is much lower in either scenario, it might be due to the presence of protein, phenol, or other pollutants that absorb heavily at/near 280 nm. DNA samples were diluted to a working concentration of 50ng/µl after quantification.

3.6.6 Amplification of DNA using Polymerase Chain Reaction

The amplification reaction mixture was produced in 0.2 ml thin-walled dome cap PCR tubes using the following components. Agilent technologies Thermocycler (Thermo fisher scientific) was used for PCR. ITS 1 and ITS 4 primers were utilised in this investigation. The reaction mixture has a total volume of 20 µl. The reaction mixture components are as in the following table.

Table 1: Primer component required to PCR reaction per sample

S. no.	Component	Quantity(µl)
1	Nuclease free water	6
2	Master mix	10
3	Primer ITS 1 (Forward)	2
4	Primer ITS 4 (Reverse)	2

3.6.6.1 List of primers

Details of primer used in molecular analysis of NTF isolates

Oligo name	Sequence (5' ……. 3')	Length	M. wt	Tm (^{0}C)
ITS 1	CTTGGTCATTTAGAGGAAGTA	21	6500.25	53
ITS 4	TCCTCCGCTTATTGATATGC	20	6033.90	53

The reaction mixture was given a quick spin to thoroughly mix all of the components before being placed in the gradient thermal cycler with the PCR tubes containing the reaction mix.

3.6.6.2 PCR amplification:

The PCR amplification for ITS 1 & ITS 4 were performed according to the protocol suggested by White et al. (1990) with slight modifications. The amplification conditions are presented in the following table.

Table 2: Steps in PCR reaction

S no.	Steps	Temp(^{0}C)	Time	Cycle number
1	Initial denaturation	96	3min	1
2	Denaturation	95	45sec	35
3	Annealing	53	1min	
4	Extension	72	1min	
5	Final extension	72	7min	1
6	Final hold	4	infinite	-

The PCR products were stored at 4˚C for short periods and at -20˚C for long duration.

3.6.6.3 Agarose Gel Electrophoresis of PCR Amplified Products

ITS1 and ITS4:

The PCR products were electrophorized on a 3% agarose gel using an Electrophoresis machine. 3.0g of agarose was weighted and added to a reagent flask containing 100 ml of 1X TBE buffer, which was thoroughly mixed.

In a microwave oven, the components were boiled with periodic mixing. The procedure was repeated until the agarose completely melted and the solution became completely transparent. The gel casting tray was cleaned with water and ethanol. When the boiling agarose had sufficiently cooled, 2 µl of ethidium bromide was added to the molten agarose, properly mixed, and poured onto the gel cast tray. The combs were placed in the gel casting tray's slots and allowed to firm at room temperature for 20-30 minutes. There was great care taken to ensure that no air bubbles were present. The gel was subsequently transferred to an electrophoresis apparatus with 1X TAE buffer. Before loading on the gel, PCR amplified products were combined with 1/6th volume of gel loading dye (40% sucrose and 0.25% bromophenol blue). The PCR products were then placed into the wells. The electrodes were then linked to a power supply. The samples were run for 60-90 minutes at 80 V. To assess the size of amplified fragments, a 100bp ladder was added to one well for each ITS1 and ITS4. The DNA fragments were then documented with a UV-transilluminator and recorded with a gel documentation system, which was then saved for future scoring and permanent records.

3.6.6.4 Molecular Identification

Molecular identification of all the isolates were carried out based on conserved ribosomal inverse transcribed spacer (ITS) region. Amplification for the ITS regions was

done between 18S rRNA and 28S rRNA using universal primer pairs ITS1 & ITS4 The amplified PCR products of all the isolates were partially sequenced (18S rRNA) from outsourcing. The results of sequencing were received in FASTA format. The FASTA sequences of amplified PCR products were identified through following procedure

- In NCBI – BLAST (https://blast.ncbi.nlm.nih.gov) website, nucleotide blast option was selected.
- In query sequence box FASTA sequence of a sample was placed for all the isolates one by one in the organism column then selecting BLAST option.
- The results of Nucleotide BLAST for all isolates were then analysed on the basis of percent identity and query cover of the samples with closely related species.

The closely related species showing a high query cover and a high percent identity with thesample were considered as the fungus isolated from the soil confirming the molecular identification of all the isolates.

3.7 Assay of the nematophagous ability of different nematode trapping fungi against *Meloidogyne javanica* and *A. triticii*

The approach outlined by Belder and Jansen (1994) was used to explore the interaction between nematode trapping fungi and *Meloidogyne javanica* and *A. triticii* in dual culture. 5 mm hyphal discs of every species were collected from the outer border of a 15-day-old culture and inoculated onto 50 mm Petri plates with corn meal agar medium (0.4% maize and agar, 3mm thickness). Inoculated Petri plates were incubated in the dark at 27^{o}C. Fungal discs were aseptically removed after 8 days of incubation. A drop of sterile distilled water containing 200 freshly hatched and completely washed *Meloidogynejavanica* (J2) and *A. triticii* were dropped into full grown cultures of all nematode trapping fungi using a dropper. Petri dishes were incubated at 25^{o}C, and observations of constricting rings and sticky traps in 1.66 mm2 fungal growth areas in response to *M. javanica* (J2) and *A. triticii* were made under a research microscope on a daily basis up to 5 days. After nematode inoculation, data on the number of traps in 1.66mm^2 regions were collected from the centre, middle, and peripheral of the fungal development, and the average number of trapped nematodes on the surface or deep into the medium wererecorded. The number of captured nematodes of all fungal species was recorded every dayfor five days, and the percentage of captured nematodes was computed. As replicates, five Petri dishes were employed. The interaction experiment was repeated twice, and pooled data were analysed using C.R.D, two

factor analysis.

3.8 Assessment of *In vitro* mycoparasitic ability of nematode-trapping fungi against different isolates of *R. solani*

The dialysis membrane approach established by Nordbring Hertz (1983) was used to investigate the mycoparasitic capability of several types of nematode-trapping fungi against *R. solani* causing sheath blight of rice and web blight of Mung bean. Washed and sterilized dialysis membranes (30 by 30 mm) were placed on the surface of LNM medium in 35 mm Petri dishes. The LNM was made up of the following ingredients: 1.0 gKCl, 0.2 g MgSO4, 0.88 mg ZnSO4 7H2O, 3.0 g FeCl3 6H2O, 0.2mg thiamine HCl, 0.005 mg biotin, 0.1g L-phenylalanine-L-valine, 10g agar, 1000ml distilled water, pH6.5). Each membrane was administered with a 5 mm disc of fungal growth of various nematode-trapping fungi (*A. conoides, A. thaumansia* isolates, *M.sphaeroides, A. Musiformis, A. oligospora* isolates, *D. phymatopaga*, and *D. brochopaga*) and *R. solani* infecting rice and mung bean. Agar discs of each nematode trapping and *R. solani* were separated by 10 mm. The average number of coils created by each nematode-trapping fungal species around the hyphae of *R. solani* was evaluated under a light microscope in a 1.66 mm2 region (100 x magnifications) at 24, 48, 72 and 96 hours after the hyphal interface of each nematode-trapping fungi species and *R. solani* isolates. In five replications, the experiment was performed two times. The experiment was laid out in a Complete Randomized Design with five replications.

3.9 Statistical Analysis

Each experiment was carried out twice. The data from the laboratory experiment was examined using a completely randomized design (CRD). $p \leq 5$ was used to compare the data.

Chapter-IV

RESULTS

4.1 Isolation and characterization of nematode-trapping fungi

During the routine isolation of nematode-trapping fungi from the horticultural and agricultural soil of two district of Bundelkhand, Uttar Pradesh, different species of nematode-trapping fungi were appeared isolated in Petri dishes containing Corn Meal Agar and Rabbit Dung Agar media (Table-3). The nematode-trapping fungi possessing different types of conidia, conidiophores and trapping organs with trapped nematodes on agar plates were observed under the compound microscope. Conidia production in beautiful arrangement on tall or small conidiophores were found in culture plates. The nematodes were trapped at head, tail and middle body region by adhesive trapping structures of nematode trapping fungi. Morphological examination of conidia, conidiophores, hyphae, chlamydospores were done and size of each structure were measured (Table-4). Each species of nematode-trapping fungi was identified on the basis of morphology of conidia, conidiophores, hyphae, with the help of relevant literatures described by various authors (Drechsler (1937, Duddington,1955, Barrron, 1977, Cooke and Godfrey,1964). Based on the morphology of isolated nematode- tapping fungi, these fungi were identified as *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys thaumasia 1, Arthrobotrys thaumasia 2, Arthrobotrys conoides, Dectylellina phymatopaga, monacrosporium sphaeroides, Arthrobotrys oligospora 1 and Arthrobotrys oligospora 2.*

Table 3- Species of nematode-trapping fungi isolated from soil of different location of Banda, Uttar Pradesh.

S. No.	Isolated species of Nematode-Trapping fungi	Locations of soil samples collected for isolation of nematode-trapping fungi.
1	*Arthobotrys musiformis*	Palahari village, Banda
2.	*Arthrobotrys conoides*	Leaf litter beneath the neem tree, Baberu
3	*Arthrobotrys thaumasia* 1	Rhizospheric zone of guava, BUAT Banda
4.	*Drechslerella brochopaga*	Aonla orchard Baberu and Mango orchard Banda.
5.	*Monacrosporium sphaeroides*	Palahari village, Banda
6.	*Drechslerella phymatopaga*	Rhiozospheric zone of aonla BUAT, Banda
7.	*Arthrobotrys thaumasia* 2	Rhizospheric zone of guava, Mahoba
8.	*Arthrobotrys oligospora 1*	Rhizospheric zone of citrus, Kamasin.
9.	*Arthrobotrys oligospora 2*	Leaf litter beneath neem tree near admin block BUAT, Banda

4.1.1 *Arthrobotrys musiformis*

On corn meal agar medium (CMA), the mycelium of *Arthrobotrys musiformis* was found spreading, hyphae hyaline, septate, mostly 2-8µm by end often give rise to horseshoe-like hyphal arches and loops in presence of nematodes. Conidiophores were hyaline, septate, erect not branched below, 148-445µm high, 5-9µm wide at the base, 2.5- 4.0µm near the tip were borne on simple branched sterigmata mostly 2-3µm wide and 3- 9µm long usually 3-10 conidia in the loose capitate arrangement. Conidia of *Arthrobotrys musiformis* were colourless, ellipsoid, or slightly bent, broadly rounded at the wider distal end tapering toward the slightly protruded base 21-43 µm long. 7.5-12.5µm wide. Chlamydospores observed in the old culture of *Arthrobotrys musiformis* in corn meal agar media. Frequent formation of chlamydospore were observed in the old cultures of *A. musiformis* in corn meal agar medium. Chlamydospores are globose or less frequently in mostly 18-22.0µm in diameter. (Figure-1)

4.1.2 *Arthrobotrys conoides*

Dreshsler originally described this fungus as *Arthrobotrys conoides* in 1937 and this

is the current name of this fungus. *Trichothecium pravicovii* Soprunov and *Arthrobotrys tortor* Jarow are the synonym of this fungus. On corn meal agar medium, the mycelium of the *Arthrobotrys conoides* was found spreading; hyphae were septate, hyaline measuring 1.5-5µm wide. Conidiophores were hyaline, septate, erect, not branched, 4-6.5 µm wide at base and 3 to 4 µm at the tip. The heights of the conidiophore were 160-350 µm on which conidia were borne in capitate arrangement. Conidia hyaline, obconial, flattened at base, broadly rounded at the tip, usually perceptibly constricted at septum, 23-35 µm long and 7-12.5 µm wide. In presence of nematodes, the hyphae of the fungus developed in the three-dimensional adhesive network and capture the nematodes. (Figure-2)

4.1.3 *Arthrobotrys oligospora* isolates

The mycelium is found to be spreading, dense, vegetative hyphae hyaline, septate, branched. Conidiophore single erect or curved slightly, septate, 140-360 µm long, 6-9µm wide at the Ba se gradually tapering upwards to a width of 3-4.5µm below the first node; Conidiophore usually continued development by repeated elongation and successive production of 1-6 additional denticle nodes were on forming several Conidia. Conidia 21-30 x 95-14 µm hyaline pyriform or ovoid, 1 septed, constricting at the septum broadly rounded at the apex 21-30 x 9.5-14 µm. The Basal cell is smaller than the digital cell. Chlamydospores are globose and thick walled 18-22 µm. Capturing nematodes by means of adhesive three-dimensional network. (Figure-3)

4.1.4 *Monacrosporium sphaeroides*

Monacrosporium sphaeroides is first reported by Drechsler. Mycelium isspreading, hyaline hyphae branched septate and mostly 2-5 µm wide. Conidiophore hyaline, erect, containing 1-7 cross walls, commonly 5.5-7.5 µm wide at the base, tapering gradually upwards to a distal width of 1.5-2.5 µm high, unbranched and bearing single conidium at the apex. Sometimes 1-5 conidia formed on 10-40um long branch near the conidiophore apex. Conidia are hyaline, broadly fusoid or elongate-ellipsoidal orobvoid, mostly 38-62 µm long and 17-32 µm wide, commonly divided by cross-walls into3-6 cells one of which greatly exceeds the other in length and width; the large cell somewhat varies in position, but occurring most often in middle position in the especially distinctive large, broadly fusoid, Quadri septate conidia, frequently 54-75 um long, and 23-35 µm wide, that are produced singly on nematode-infested substratum. Capturing nematodes by means of three-dimensional adhesive network. (Figure-4)

4.1.5 *Arthrobotrys thaumansia* isolates

Arthrobotrys thaumansia Is first time discovered by Drescher. Mycelium is spreading vegetative hyphae Hyaline Septate branched 3-6 µm wide. Conidiophore hyaline erect septate 210-420 µm long, 3-7 µm wide at base, gradually tapering upwardsto a width of 2.5-3.5 µm at the apex simple or often somewhat branched near the apex andthey are born on blunt sterigmata mostly 2-10 µm long and 2-3 µm wide usually 3 to 15 rarely up to 25 conidia in loose capitate arrangement conidia. Conidia hyaline top shaped rounded at the apex tapering towards the frequently somewhat protruding truncate base 34-47 µm x 16.6-23 µm, mostly 3 septate. Chlamydospores are observed and capture the nematodes by 3-dimensional network. (Figure-5)

4.1.6 *Drechslerella brochopaga*

Drechslerella brochopaga was first reported by Charles Drechsler in 1937 as *Dactylaria brochopaga.* Based on the new classification, *Drechslerella brochopaga* Drechsler is the current name of this fungus. The mycelium of *Drechslerella brochopaga* on corn meal agar medium was spreading, hyphae hyaline, septate, 1.9- 4.6 µm wide, conidiophores hyaline, septate, erect, 80-350 µm, more typically 200- 350 µm high, 3- 6µm wide at the base, tapering gradually upward to a width of 2.2- 3.4µm near the tip, there bearing on short blunt sterigmata 3- 12, mostly 3- 8 conidia in the beautiful radiatingcapitate arrangement- or less often and less typically producing up to 14 conidia in more scattered, irregularly racemose arrangement. Conidia hyaline, straight or slightly curved, cylindrical or elongate ellipsoidal, broadly rounded at the apex, usually tapering noticeably toward the somewhat truncate base, 24-36 µm long, 5-9µm wide, and containing 3 cross walls into 4 cells. In presence of nematodes, the hyphae of the fungus developed constricting rings. Constricting rings produced on the conidia and on thehyphae were found 20 to 34 µm in outside diameter and composed of three arcuate cells. The constricting rings capture and kill free living and plant parasitic nematodes at head, tail, and middle body region and grow inside the body region of nematodes. Constricting rings produced on conidia (conidial traps) were also found to capture and kill the nematodes. (Figure-6)

4.1.7 *Dactylellina phymatopaga*

This fungus was reported by Drechsler in the year 1954 and named it *Dactylellina phymatopaga. Dactylellina phymatopaga* (Drechsler) Yan Li is now valid name of this

fungus. The other synonym of this fungus is *Monacrosporium phymatopagum* (Drechsler) Subram.; *Golovinia phymatopaga* (Drechsler) Mekht. and *Gamsylella phymatophaga* (Drechsler) M. Scholler, Hagedorn & A. Rubner. Mycelium of this fungus on corn meal medium was found often scanty, hyphae colour less, branched, septate mostly 1-2.5 µm wide and produce adhesive protuberances at intervals of 10-50 µm. Adhesive protuberances were found sessile, obovoid or prolate ellipsoid, mostly 6.0-9.0 µm long and 2-3 µm wide at the base and 4.0 to 5.0 µm in greatest width. Conidiophores hyaline, erect, containing 4-9 septa, 160 to 375 µm tall, 4.5-7 µm wide at the base, tapering gradually upward about 2-3.5 µm wide at tip on which a single conidium is borne. Conidia hyaline, five celled with larger middle cell, usually spindle shaped, truncate and rather narrow at the base, rounded at the distal end mostly 42-56 µm long and 22-25 µm wide. (Figure-7)

4.2 Molecular Characterization of nematode trapping fungi

After morphological characterization, the molecular characterization was done by amplification of ITS region of isolated spp. Of nematode trapping fungi by primers ITS 1 & ITS 4. The amplified PCR product were sequenced from outsourcing and obtained sequence of each *spp.* of nematode trapping fungi were analyzed by BLAST (Basic Local Alignment Search Tool) search of NCBI database. Based on the sequence similarities of nematode trapping fungi with existing database on NCBI, these nematode trapping fungi were molecularly characterized as two strains of *Arthrobotrys oligospora* 99.75% similarity with *Arthrobotrys spp. ,* two strains of *Arthrobotrys thaumasia,* 95% similarity with *A. thaumasia* one strain of *Arthrobotrys conoides* 95% similarity with *A. conoides, Arthrobotrys musiformis* 96% similarity with *A. musformis, Drechslerella brochopaga* 95% similarity with *D. brochopaga* and *Dactylellina phymatopaga* 95% similarity with *Monacrosporium phymatopaga* however one isolate morphologically identified as *Monacrosporium sphaeroides* have 99% similarity with *A. conoides* and 97% similarity with *M. sphaeroides.* This difference may be due to mixing of recombination betweenboth the fungi in nature or may be due to minute mixing of the culture with *A. conoides.* Further studies may be needed for the final taxonomic resolution of *M. sphaeroides* characteristics in present investigation. The similarity coefficient values among all the isolates are given in table 5. The ITS sequences of all the nematode trapping fungi are given below.

4.2.1 ITS region sequences of *Drechslerella brochopaga*

AAATAAGATTCGTAGGTGACCTTGCGGAAGGATCATCACCAAAACAGAAGCA
ATACTCGGCTTACGGCCTATGAAAGCTATCAACCTTATTGTGAACCAAACCTTT
CTTTTCGCTTCGGCAGCAGCGGCGATCCCGCCGCGTCAGCCTGCCGCTAGCACCCTTGA
AAACTTGCTGTATCTCATGTCTGAACACGAATATTTTGAATTCAATCAAAACTTTCAAC
AACGGATCTCTTGGTTCCCGCATCGATGAAGAACGCAGCGAAACGCGATAGTTAATGT
GAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCATATTGCGCCCATTGGTATT
CCATTGGGCATGTCTGTTTGAGCGTCATTTCAAACCCTCGACTTTGGTCGGTATTGAGC
TGGCTTTGCGGGTGCGAACCCAGGCCGGTTTTAAAGTGGTAGGCTTTGCTGTCTGCTGC
TCCAAACCAAAAACATAGTAAACTGTACTTGTTGATGGTGGGGTGGCTCCAGCCTTGA
AAATGACACTTTGTGGTTTGACCTCAGATCAGACAAGGATACCCGCTGAACTTAAGCA
TATCATAAAGCC GGGAGGAAAGCC

4.2.2 ITS region sequences of *A. thaumasia* 1

AACTAAGAAACCTTTTCTCGGGTAGTTCGTAGGTGACCTGCGGAAGGATCATT
ACCAAAGGAGTGAGAAATCACTACCTCTTACCTGCTACGGTGGCCCCTTCGGG
CCGCTGACTGGTCAACCCTTTGTGAACCAAAAAAAACCTTTTCGCTTCGGCAGC
TGGGCCTGACCGCCTGTCAGCCTGCCGCTAGCACCCAACCGAAAAACCTGTTG
TCAAAACATTTGTCTGATAACCAAATTTTCGAATGAAAATCAAAACTTTCAAC
AACGGATCTCTTGGTTCCCGCATCGATGAAGAACGCAGCGAAACGCGATAGTT
AATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGC
CCATTGGTATTCCTTTGGGCATGTCTGTTTGAGCGTCATTACAACCCCTCAGCT
AACGCTGGTTTTGAACCGGGACAGGTTGACACCCGCACCGGTTTTAAAGTTGT
AAGCTCTGCTGGCTGCTCTGCCCCAACCGGAACATAGTAAAACTACTACTTGTT
AAGGCGAGGCGAAGCGGTACGGCCTGAACAAAACCTACCCATTTTTCTAAGGT
TTGACCTCAGATCAGACAAGGATACCCGCTGAACTTAAGCATATCATAAAAGG
CGGGAGGAGGAA

4.2.3 ITS region sequences of *A. musiformis*

CTTGACTCGGTTTTTTGTTCGGTAGTTTCGTAGGTGACCTGCGGAAGGATCATA
ACCAATACAGCGGGAGGCCGCGAGGCCTACTTGTACTGCCTGTTTGGCCGCCT
TAATGCGCGGTCAACTGGTTAACCCTTTGTGAACCAAAAAACCTTTCGCTTCGG
CAGCTGGGCCCTGACCGGCCTGTCAGCCTGCCGTTAGCACCCTTCAAAAACTT
GTTGTCAAAACATTGTCTGATAACCAAATTTTCGAATGAAAATTAAAACTTTCA
ACAACGGATCTCTTGGTTCCCGCATCGATGAAGAACGCAGCGAAACGCGATAG
TTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCG

CCCATTGGTATTCCTTTGGGCATGTCTGTTTGAGCGTCATTACAACCCCTCAGC
TAACGCTGGTTTTGAACCCGAACGGCCTCGTGCCGCACCGGTTTTAAAGTTGTA
AGCTCTGCTGGCCGCTCTGCCCCAACCAGAACATAGTAAGCAACTACTTGTTA
GGGTGAAGGTGAACGGTTACGGCCTGAACAAAACCTACTTCTCAAGGTTTGAC
CTCAGATCAGACAAGGATACCCGCTGAACTTAAGCATAT

4.2.4 ITS region sequences of *A.conoides*

GGGTCAGTTCCGTAGGTGACCTGCGGAGGATCATTACCAATACCAGCCTGCCG
GCTGCCGCTGTGGTTGTTAAGTCAACAGCACGGTTTTCCGGTCAGGTGAACCA
GTACCACCTGTGCTTCGCATGGGTACCTGGGTCAACCCTTTGTGAACCAAAAC
AAACCTTTCGCTTCGGCAGCGGGGCCCTAAACCAGCCCGTCAGCCTGCCGCTA
GCACCAAACAAAACAAAACTTGTTGTATAAACATTGTCTGACAACCAAATTTT
CGAATGAAAATCAAAACTTTCAACAACGGATCTCTTGGTTCCCGCATCGATGA
AGAACGCAGCGAAACGCGATAGTTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAGTCTTTGAACGCACATTGCGCCCATTGGTATTCCTTTGGGCATGTCTGTTT
GAGCGTCATTACAACCCTCAGCTAACCGCTGGTTTTGGACCCGAACGGCTTGA
CTGCCGCGCAGGTTTTAAAGTTGTAAGCTCTGCTGGCCGCTCTGCCCCAACCGG
AACATAGTAAGCACTACTTTTGTTAAGGTGAAGCAGAACGGTACGGCCTGTAA
CAAAACCTACCCTTTTTCTCAAGGTTTGACCTCAGATCAGACAAGGATACCCGC
TGAACTTAAGCATATCATAAAGCGGGAAGGAAGAAG

4.2.5 ITS region sequences of *A. oligospora* 2

GAGGGGCTTGAGTAGATCCTTCCATAGGTGACGCTCACGAGAAGCCTTTGTGG
CCCCGCAAGGGGTACCTGCCGCGACTGTAAACAATGAAAGCGGGTATTAAATC
GCAAGTCAGCTGTTGCTGGCTACACTTTCGAATTGCGGGGAAATCCTAAAGCC
TGCCTCTACCAACCATTTGGAGAAATCTGATTGGGGCCTGTGTTAACAGCATA
GGGTGTGGTAATAACGAGGCGGGATACGTCAGCTTGGGCTGGCATCATGGGCA
ATCCGCAGCCAAGTCCCTAAGGCATCAGCTATGGGAAAGGTTCACAGACTAAG
TGGAAGTGGGCTGGATCTGTTTCAGCTTAAGATATAGTCGGGCTCTATGCGAA
AGCACAGAGGAGTCATTCTAACCGTTCCGTAGGTGAACCTGCGGAAGGATCAT
TACCAAAAGGCGAGAAATCGCTTTGCTACCTGCTCGGTGGCCCTACCCGGGCT
GCTGACTGGTTAACCCTTTGTGAACCAAAAAACCTTTCGCTTCGGCAGCTGGGT
CCCGAGAGGGCCCCGTCAGCCTGCCGCTAGCACCTAATTCAAAAACCTGTTGT
CAAAACATTTGTCTGATAACCAAATTTTCGAATGAAAATCAAAACTTTCAACA
ACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAACGCGATAGTTA

ATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCC
CATTGGTATTCCTTTGGGCATGTCTGTTTGAGCGTCATTACAACCCTCAGCTAA
CCGCTGGTTATGAACCTGAACGGGTCTCACCCCGCACCGGTTTTAAAGTTGTAA
GCTCTGCTGGCTGCTCTGCCCCAACCGGAACATAGTAAGCAACTACCTTGTTCA
CAGGTTAAGCGGAACGGTACGGCCTGAACAAAACCTACCCTCTCTAAGGTTTG
ACCTCAAATCAAACAAGGATACCCGCTGAACTTAAG

4.2.6 ITS region sequences of *A.oligospora* 1

AAAAGGGCTTGAAGTAAGATCCTTCCATAGGTGACGCTCACGAGAAGCCTTTG
TGGCCCCGCAAGGGGTACCTGCCGCGACTGTAAACAATGAAAGCGGGTATTAA
ATCGCAAGTCAGCTGTTGCTGGCTACACTTTCGAATTGCGGGGAAATCCTAAA
GCCTGCCTCTACCAACCATTTGGAGAAATCTGATTGGGGCCTGTGTTAACAGC
ATAGGGTGTGGTAATAACGAGGCGGGATACGTCAGCTTGGGCTGGCATCATGG
GCAATCCGCAGCCAAGTCCCTAAGGCATCAGCTATGGGAAAGGTTCACAGACT
AAGTGGAAGTGGGCTGGATCTGTTTCAGCTTAAGATATAGTCGGGCTCTATGC
GAAAGCACAGAGGAGTCATTCTAACCGTTCCGTAGGTGAACCTGCGGAAGGAT
CATTACCAAAAGGCGAGAAATCGCTTTGCTACCTGCTCGGTGGCCCTACCCGG
GCTGCTGACTGGTTAACCCTTTGTGAACCAAAAAACCTTTCGCTTCGGCAGCTG
GGTCCCGAGAGGGCCCCGTCAGCCTGCCGCTAGCACCTAATTCAAAAACCTGT
TGTCAAAACATTTGTCTGATAACCAAATTTTCGAATGAAAATCAAAACTTTCAA
CAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAACGCGATAGT
TAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCG
CCCATTGGTATTCCTTTGGGCATGTCTGTTTGAGCGTCATTACAACCCTCAGCT
AACCGCTGGTTATGAACCTGAACGGGTCTCACCCCGCACCGGTTTTAAAGTTGT
AAGCTCTGCTGGCTGCTCTGCCCCAACCGGAACATAGTAAGCAACTACCTTGTT
CACAGGTTAAGCGGAACGGTACGGCCTGAACAAAACCTACCCTCTCTAAGGTT
TGACCTCAAATCAGACAAGGATACCCGCTGAACTTA

4.2.7 ITS region sequences of *M. sphaeroides*

CGGTAAGGTTGAGTAGATCCTTCCATGGGTGACGCTCACGAGAAGCCTTTGTG
GCCCCGCAAGGGGTACCTGCCGCGACTGTAAACAATGAAAGCGGGTATTAAAT
CGCAAGTCAGCCGGTTCGACCGGCTGGCCACACTTTCGAATTGCGGGGAAACC
CTAAAGCCCGCCTCTACCAACCAGCTGGGGAAATCCGGCTGGGGCCTGTGTTA
ACAGCACGGGGTACGGTAATAACGAGGTGGGATATGTCAGCCGCTGGCTGGC
ACCATGGGCAATCCGCAGCCAAGTCCCTACCGCTCGACAGCTATGGGAAAGGT

TCACAGACTAAGTGGAAGTGGGCTCGGGTCAAGAATCTGAGCTTAAGATATAG
TCGGGCTCTGTGCGAAAGCGCTGAGGGGTCACATCTCTCCAACCGTTCCGTAG
GTGAACCTGCGGAAGGATCATTACCAAAAAGCGAGCGATCGCTTTCTACCGCT
CGGTGACCCTCTGGGTCGCCGACTGGTCAACCCTTTGTGAACCAAAAAAAACC
TTTCGCTTCGGCAGCTGGGCCTTACCCGCCCATCAGCCTGCCGTTAGCACCTTT
CCAAAAAACCTGTTTGTCAAAACATTGTCTGATGACCAAATTTTCGAATGAAA
ATCAAAACTTTCAACAACGGATCTCTTGGTTCCCGCATCGATAAAGAACGCAG
CGAAACGCGATAGTTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTT
GAACGCACATTGCGCCCATTGGTATTCCTTTGGGCATGTCTGTTTGAGCGTCAT
TACAACCCTCAGCTAACCGCTGGTCTTGAACCTGACCGGGTAACACCGTGCCG
GTTTTGAAGTTGTAAGCTCTGCTGGCCGCTCCGCCAAACCAGAACATAGTAAG
CACTACTTGTTTGGGCGAAGCCGAGCGGTACGGCCTGAACAGAACCTACCCAT
TTTCTCTAAGGTTTGACCTCAGATCAGACAAGGATACCCGCTGAACTTA

4.2.8 ITS region sequences of *A.thaumasia* 2

CAAAGTTCGTAGGTGACCTGCGGAAGGATCATTACCAAAGGAGTGAGAAATC
ACTCCTCTTACCTGCTCGGTGGCCCCTTCGGGCCGCTGACTGGTCAACCCTTTG
TGAACCAAAAAAAACCTTTTCGCTTCGGCAGCTGGGCCTGACCGCCTGTCAGC
CTGCCGCTAGCACCCAACCGAAAAACCTGTTGTCAAAACATTTGTCTGATAAC
CAAATTTTCGAATGAAAATCAAAACTTTCAACAACGGATCTCTTGGTTCCCGCA
TCGATGAAGAACGCAGCGAAACGCGATAGTTAATGTGAATTGCAGAATTCAGT
GAATCATCGAGTCTTTGAACGCACATTGCGCCCATTGGTATTCCTTTGGGCATG
TCTGTTTGAGCGTCATTACAACCCCTCAGCTAACGCTGGTTTTGAACCGGGACA
GGTTGACACCCGCACCGGTTTTAAAGTTGTAAGCTCTGCTGGCTGCTCTGCCCC
AACCGGAACATAGTAAAACTACTACTTGTTAAGGCGAGGCGAAGCGGTACGG
CCTGAACAAAACCTACCCATTTTTCTAAGGTTTGACCTCAGATCAGACAAGGA
TACCCGCTGAACTTAAGCATATCATAAGCCGGGAGGAAAAA

4.2.9 ITS region sequences of *D. phymatopaga*

GAGTTTCCGTAGGTGACCTGCGGAAGGATCATATCCCAAAACTTTTAGCTTGTC
TGGTCGCAAGGCCGTGACGCTTCAACCCTTTGTGAACCAAAAAAACCTTTTCG
CTTCGGCAGCTGGGCCGGTTGGAGACAACCGCTCCGTCAGCCTGCCGTTAGCA
CCAACCTTCAAAACTTGCAGTCAAAAACATTGTCTGAACACCAAATTTTCGAA
TGAAAATCAAAACTTTCAACAACGGATCTCTTGGTTCCCGCATCGATGAAGAA
CGCAGCGAAACGCGATAGTTAATGTGAATTGCAGAATTCAGTGAATCATCGAG

TCTTTGAACGCACATTGCGCCCATTGGTATTCCGATGGGCATGTCTGTTTGAGC
GTCATTACAACCCTCGGCTCCAGCCGGTTTTGAGCGCGCCTGGCCATCACGGCC
CTGGCTAGCTTTAAAGTTGTAAGCTCTGCTGACCGCTCCTGCTGAACCAGAACA
ATAGTAAAATTTATTCTACTTGTTCGCAAGGCGAAGCTCTGGCGGTTTGGCCTG
AACAAAACCTACCCAACCTAAGGTTTGACCTCAGATCAGACAAGGATACCCGC
TGAACTTAAGCATATCATAAAGCCCGGAAGGAAA

4.3 Assessment of trap formation and nemtaophagous ability of *native* strainsnematode trapping fungi of Bundelkhand region

Inoculation of 200 freshly hatched second stage juveniles (J_2) of *A. triticii* and *M. javanica* into pure culture of all nematode trapping fungi in corn meal agar medium (0.4%) resulted in induction of constricting rings, unstalked knob, adhesive net and three-dimensional network on the hyphae of *D. brochopaga, D. phymatopaga, A. musiformis* and *A. oligospora2, A. oligospora 1, A. thaumasia 1, A. thaumasia 2, A. conoides, M. sphaeroides* within 24 h respectively. Number of constricting rings and adhesive traps were increased with the passage of time. Increase in the average number of trapping structures in all fungi in response to *A. triticii* in 1.66mm^2 area were higher in *D. brochopaga* followed by *A. oligospora 2*, *D. phymatopaga*, *A. oligospora 1*, *M. sphaeroides*, *A. thaumasia 1*, *A. musiformis*, *A. thaumasia 2* and *A. conoides* where as in case of *M. javanica* interaction, trapp formation is highest in *D. brochopaga* followed by *D. phymatopaga*, *M. sphaeroides, A. thaumasia 1, A. thaumasia 2, A. conoides, A. oligospora 1, A. musiformis,* and *A. oligospora 2*. In case of *A. triticii*, higher percentageof trapped nematodes were observed in *A. oligospora* 2 on 5th day of nematode inoculation per 1.66 mm^2 area of fungal growth whereas in case of *M. javanica* maximum trapping was observed in *D. brochopaga*. Second stage juveniles of *A. triticii* and *M. javanica* were freely moved in the media and captured by Adhesive 3 D network of *A. oligospora* 1, *A. oligospora* 2, *A. thaumasia 1, A. thaumasia 2, M. sphaeroides*, *A.conoides*, adhesive nets of *A. musiformis*, constricting rings of *D. brochopaga* and unstalked adhesive knob of *D. phymatopaga.* The trapped nematodes were digested completely after 48 hours of trapping by all the nematode-trapping fungi. *A. oligospora* 2 trapped and killed the highest (75.83%) J_2 of *A. triticii* whereas *A. conoides* captured only 37.83% *A. triticii* (J_2) within 120 hours after nematode inoculation. In case of *M. javanica, D. brochopaga* is found to be most predacious (75.62%) and *A. conoides* is the less predacious (45.80%). All the native strains captured and killed *A. triticii* and *M. javanica*

at head, tail, and middle body region by three-dimensional adhesive network, constricting rings, adhesive nets, sessile knobs. Results revealed that trap formation and trapping of nematodes had a strong relation for the nematode-trapping ability of all the native strains of nematode trapping fungi. The ability of production of higher number of constricting rings in *D. brochopaga* in comparison to the adhesive loops of other fungi may be attributed to the response of these fungi to the morphogenic substances produced by the nematodes. *A.oligospora* 2 and *A.oligospora* 1 are more efficient trapper of *A. triticii* and *D. brochopaga* of *M. javanica* may be due to the genetic makeup of these fungi and large size of nematode (*A. triticii)* which make them able to be more efficient nematode trapper by formation of abundant trap formation and trapping of nematodes.

4.4 Mycoparasitic efficacy of native strains of nematode trapping fungi of Bundelkhand region against *R. solani* isolates (AG-1 and AG-3) in dual culture

Hyphal interface of different species of nematode - trapping fungi with different isolates of *R. solani* resulted in the formation of infectious hyphal coils of nematode- trapping fungi around the hyphae of *R. solani* except *D.brochopaga*, *D. phymatopaga* which did not form hyphal coils to parasitized the test pathogen. Coiling was observed within 24 hours of interface of nematode -trapping fungi and *R. solani* in dual culture. Variable frequency of coiling was observed after interface of *R. solani* and nematode- trapping fungi. *A. thaumansia* 1 was found most able mycotroph on both the isolates of *R. solani* followed by *A. musiformis, A. oligospora* 2, *A. oligospora* 1, *M. sphaeroides, A. conoides*. However, the number of coils was found less in rice isolates (AG-1) during hyphal coiling (mycoparasitism) of *R. solani* by *A. thaumansia,* 1, *A. thaumansia,* 2, *A. musiformis, A. oligospora* 2, *A. oligospora* 1, *M. sphaeroides*, *A. conoides* than Mungbeanisolates (AG-3). This indicates that rice isolates had some tolerance to coiling and native isolates of nematode- trapping fungi are required for parasitism of Mungbean isolates of *R. solani* (AG-3) more aggressively.

Table 4: Morphological character of isolated strains of nematode trapping fungi

Nematode trapping fungi	Characteristic feature of fungi							
	Mycelium	**Conidial shape**	**Conidial size**	**Conidiophore length**	**Conidiophore width at base**	**Conidiophore width at tip**	**Trapping device**	**Chlamydospore**
A.musiformis	Hyaline, spreading, septate,2-8 µm wide	Hyaline, bicelled, slightly curved,rounded at wider distal end, slightly protruded base	21-43 µm x 7.5-12.5 µm	148-445 µm	5-9 µm	2.5-4.0 µm	Adhesive traps	Present
A.oligospora strains	Hyaline, spreading, septate, branched	Hyaline, bicelled, ovoid, rounded at apex.	21-30 µm X 9.5-14 µm	140-360 µm	6-9 µm	3-4.5 µm	Three-dimensional network	Present
A.thaumasia strains	Hyaline, spreading, septate	Hyaline, top shaped, rounded at the apex with protruding truncate base	34-47 X 16.6-23 µm	210-420 µm	3-7 µm	2.5-3.5 µm	Three-dimensional network	Present
A.conoides	Hyaline, spreading, septate, branched, 1.5-5 µm wide	Hyaline, obconical, flattened at the base, rounded at apex, single septate and constricted at the septum.	23-35 X 7-12.5 µm	140-350 µm	4-6.5 µm	3-4 µm	Three-dimensional network	Present
M. sphaeroides	Hyaline spreading, septate, branched, 2-5 µm wide	Hyaline, broadly fusoid or elongate-ellipsoid or obvoid, septate, 3-5 celled, large cell varies in position but mostly in median position.	38-62 X 17-32 µm	350-400 µm	5.5-7.5 µm	1.5-2.5 µm	Three-dimensional network	Present
D.phymatopaga	Hyaline, scanty,septate, 1-2.5 µmwide	Hyaline, spindle shaped truncate and narrow at the base and rounded at the distal end.	42-56 X 22-25 µm	160-375 µm	4.5-7 µm	2-3.5 µm	Unstalked knob	Absent
D.brochopaga	Hyaline, spreading, septate,1.9-4.6 µm wide	Hyaline, straight or slightly curved, elongated, rounded at apex	24-36 X 5-9 µm	80-350 µm	3-6 µm	2.2-3.4 µm	Constricting ring	Absent

Figure 1: Morphological characteristics of *Arthrobotrys musiformis*

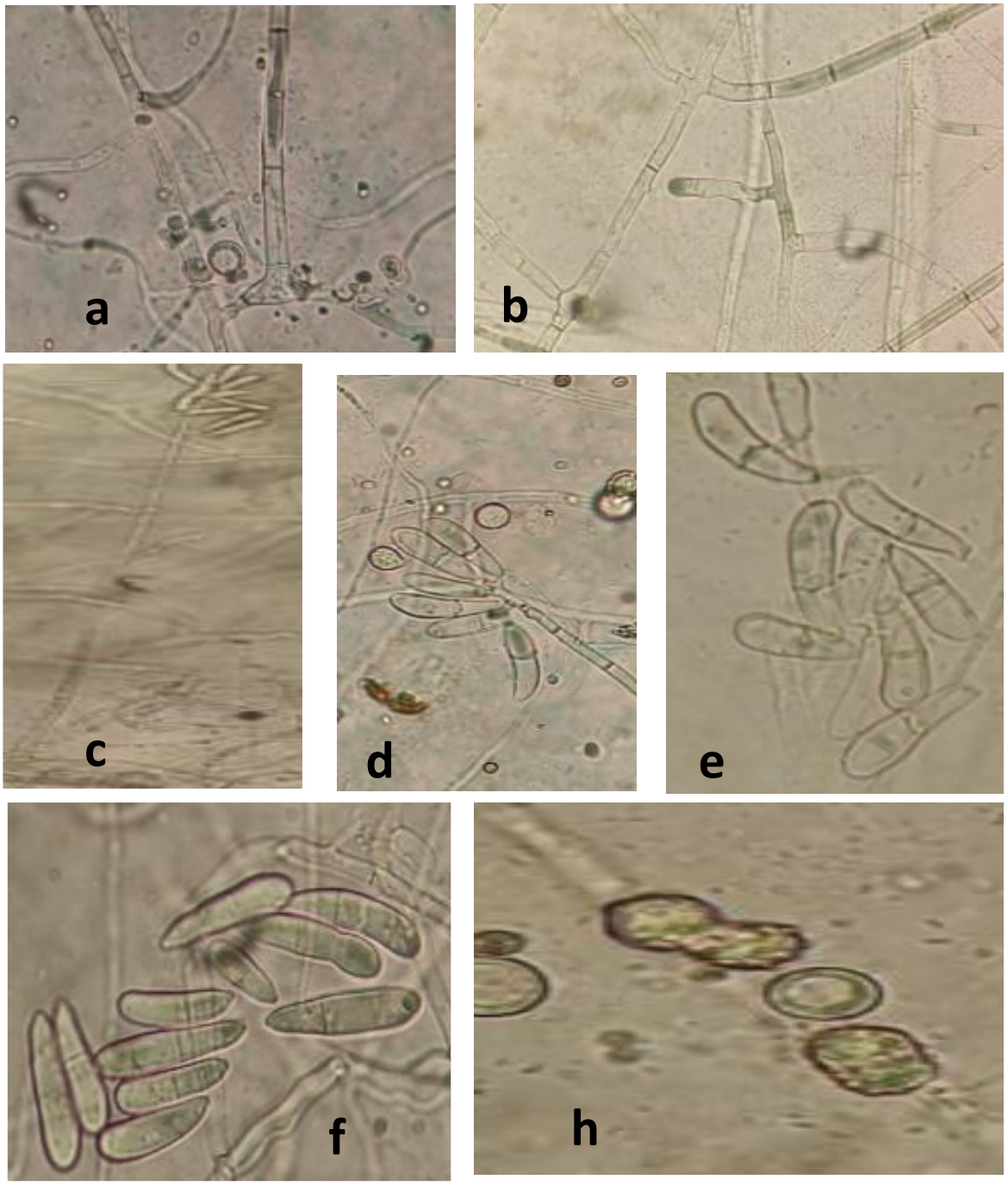


Figure : a- base of conidiophore (400x), b– hyphae(400x), c conidiophore(400x), d- tip of conidiophore(400x), e-f shape of conidia(400x), g-h chlamydospores(400x).

Figure 2: Morphological characteristics of *Arthrobotrys conoides*.

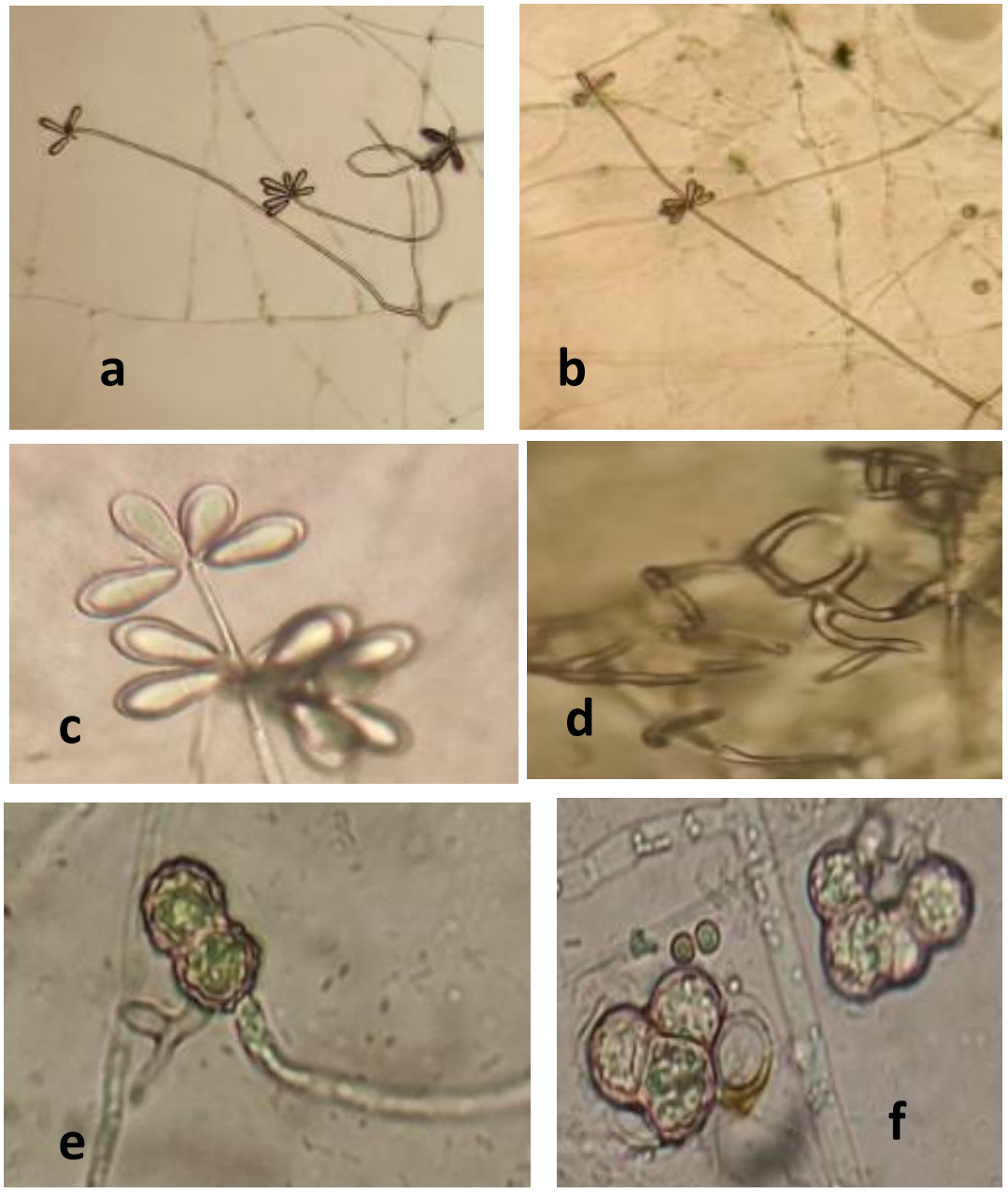


Figure : a,b conidiophores (100x), c- conidia (400x), d-three dimensional network (400x), e,f,- chlamydospres(400x).

Figure 3: Morphological characterstics of *Arthrobotrys oligospora* strains

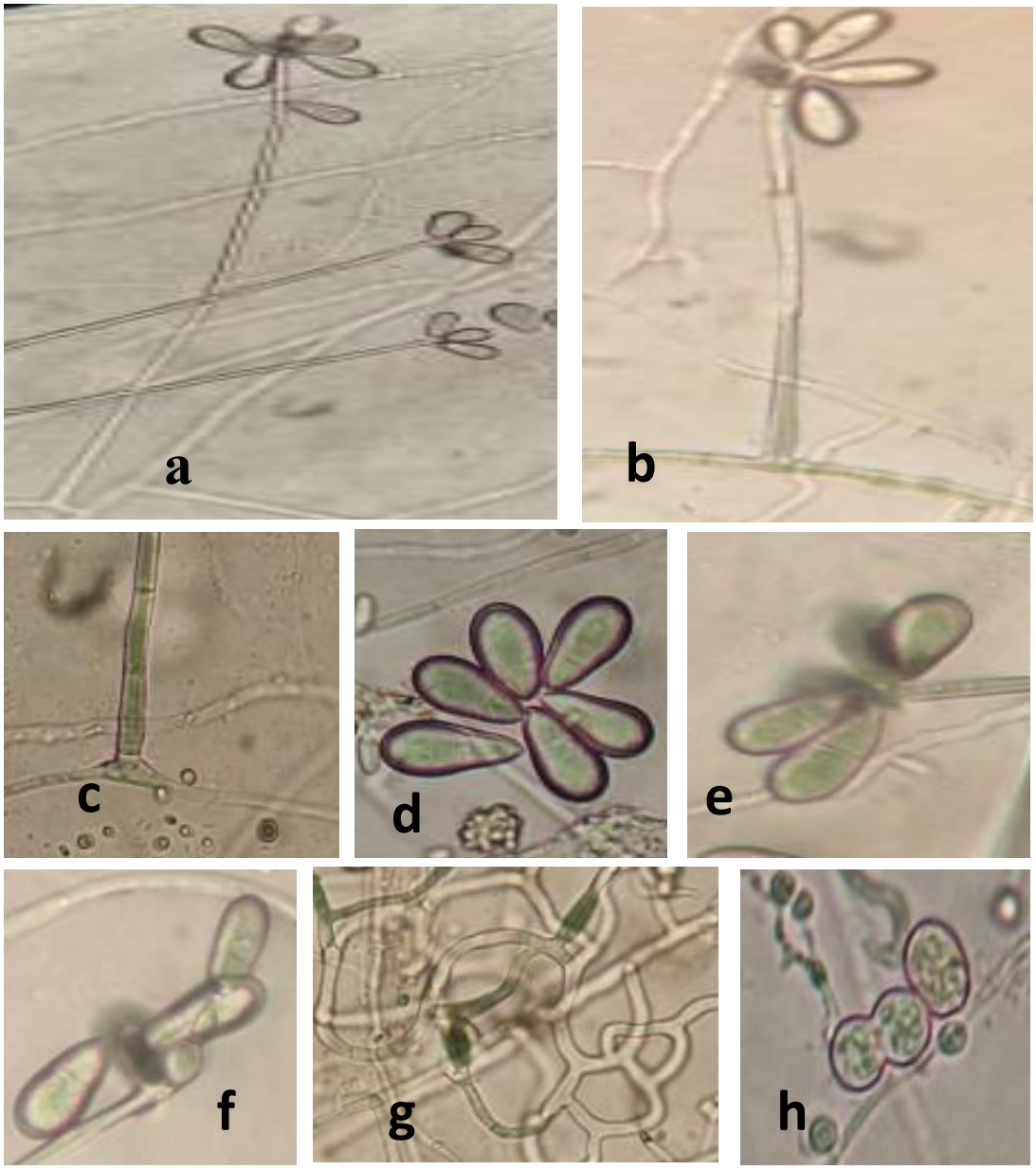


Figure: a,b- conidiophore(100x), c base of conidiophore(400x), d,e,f- conidia(400x), g- three dimensional network(400x), h chlamydospores(400x).

Figure 4 : morphological characteristics of *M. sphaeroides*

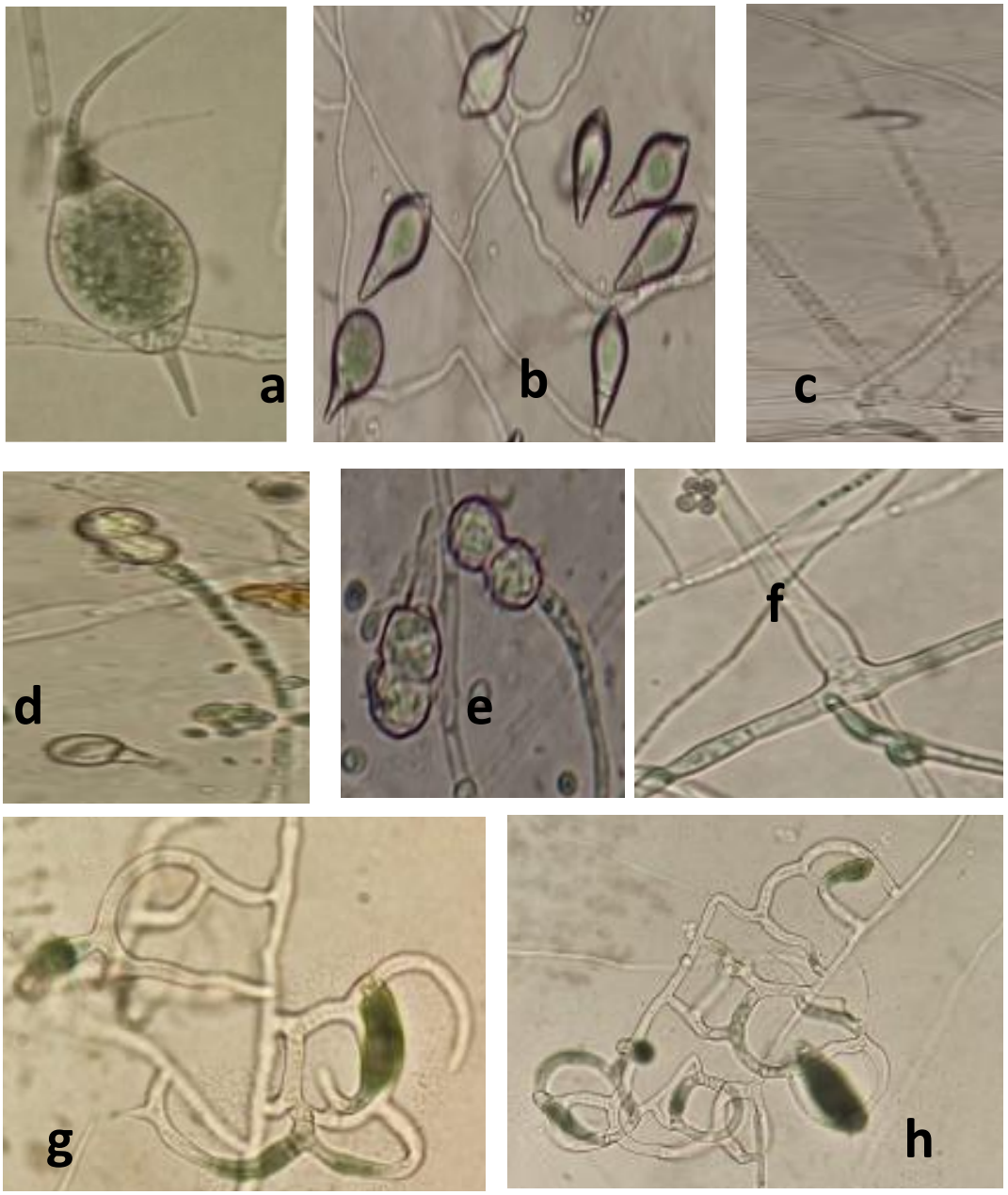


Figure: a,b- conidia(400x), c- conidiophore (400x), d,e- chlamydospores (400x), f- base of conidiophore(400x), g,h- three dimensional network

Figure5: Morphologicalcharacteristicsof *A. thaumasia* isolates

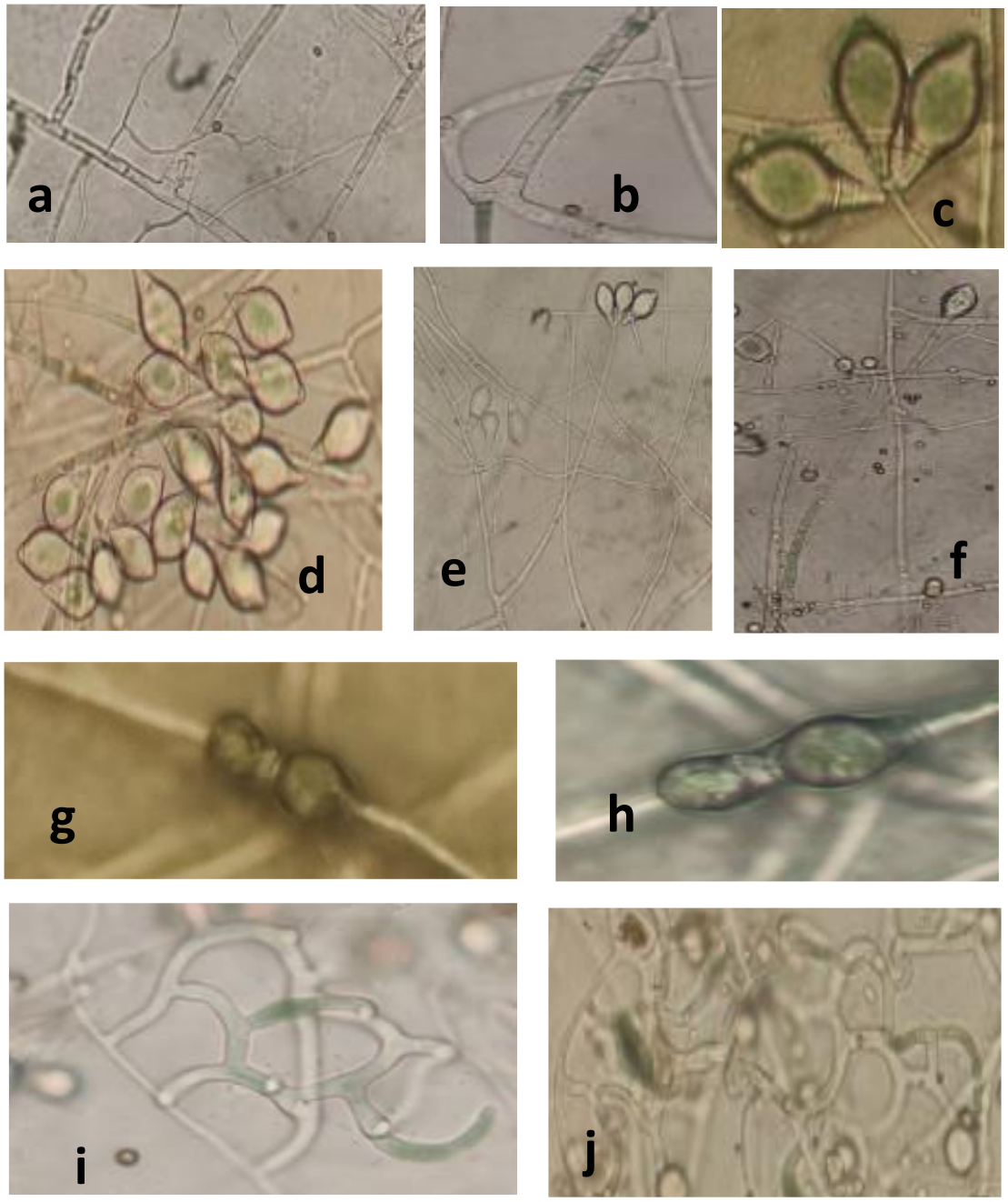


Figure 5 a- Hyphae(100x), b- base of conodiophore(400x), c,d-conidia(100x), e,f-conidiophore(400x), g,h - chlamydospores(400x) i,j - three dimensional network(400x).

Figure6: Morphologicalcharacteristicsof *D. brochopaga*

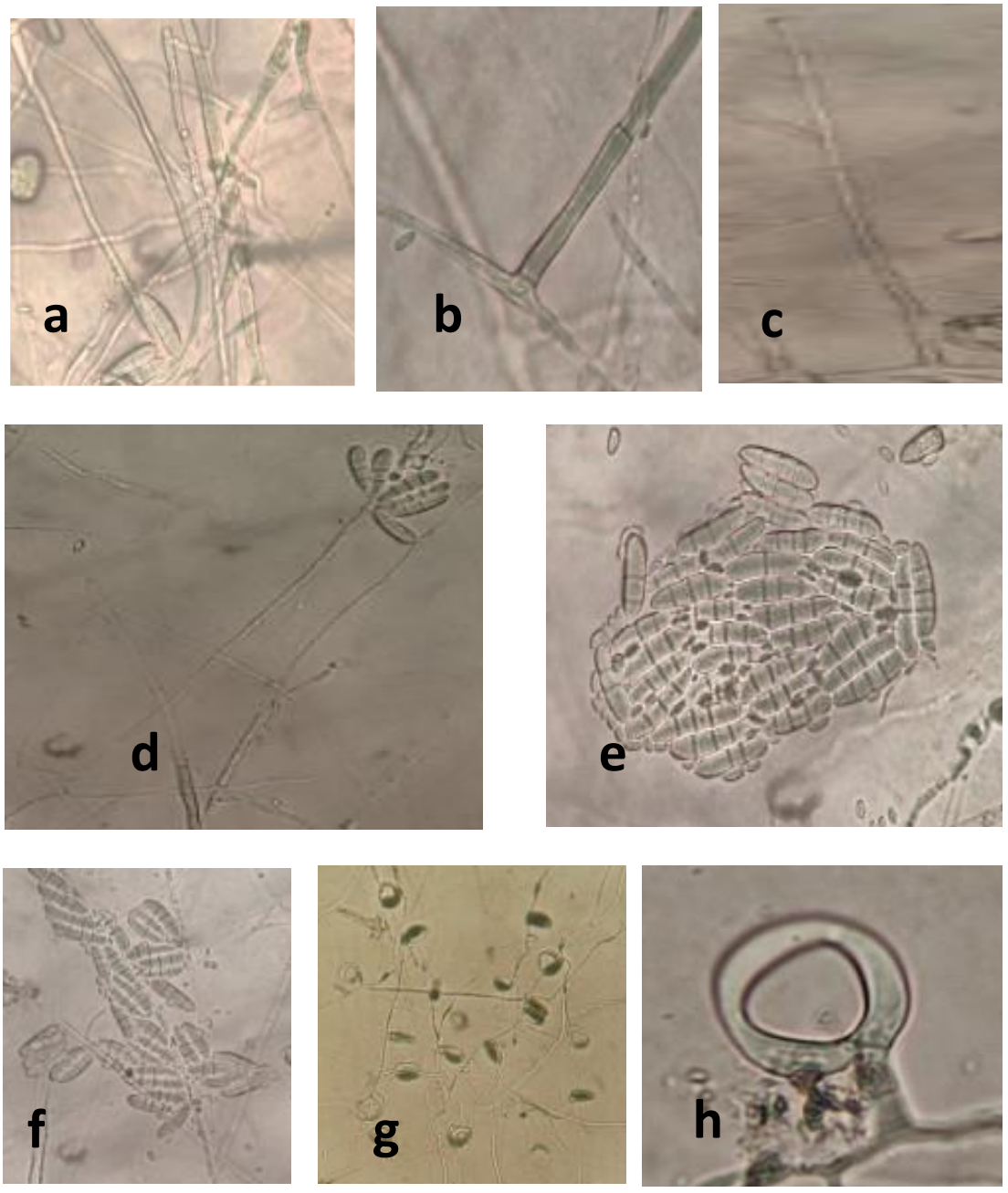


Figure: a- hyphae(100x), b- base of conidiophore(400x), c,d- conidiophore(400x), e,f- conidia(400x), g,h- constricting ring(100x , 400x)

Figure 7: Morphological characterstics of *D. phymatopaga*

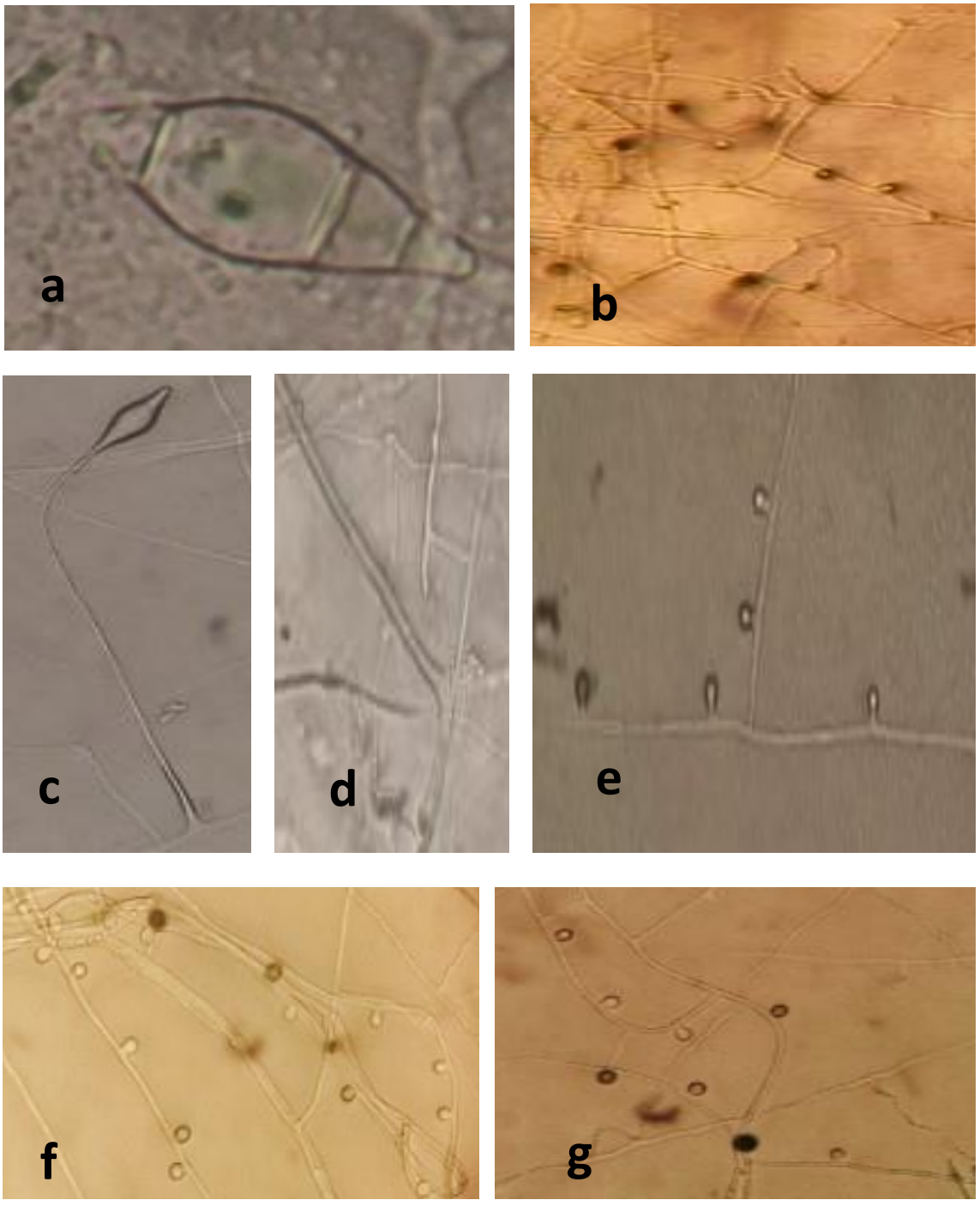


Figure : a- conidia (400x), b-hyphae (100x), c-conidiophore (400x), d-base of conidiophore(400x), e,f,g- unstalked (sessile) knob (400x),

Figure 8: Banding pattern of nine isolates of nematode trapping fungi amplified with ITS1& ITS 4 primers on agarose gel, 100 bp ladder.

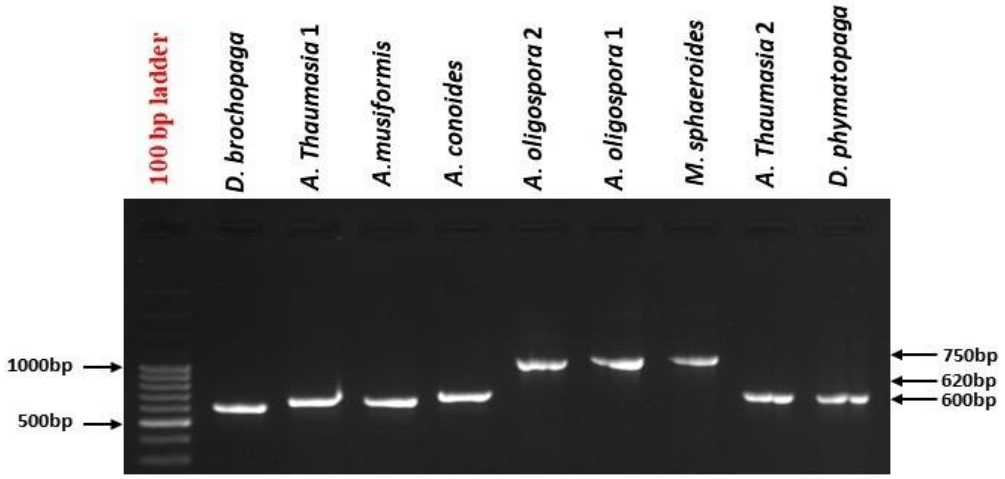


Figure 9: Phylogenetic tree analysis of all the nematode trapping fungi, (F3 ATH- *Arthrobotrys thaumasia 1,* F10 ATH2- *A. thaumasia,* F6 AO2- *A. oligospora2,* F8 AO1- *A. oligospora1,* F9 AMeg- *M.spharoides,* F4 AM- *A.musiformis,* F5 AC- *A. conoides,*F1 DB- *D. brochopaga,* F11 DPHY- *D. phymatopaga*

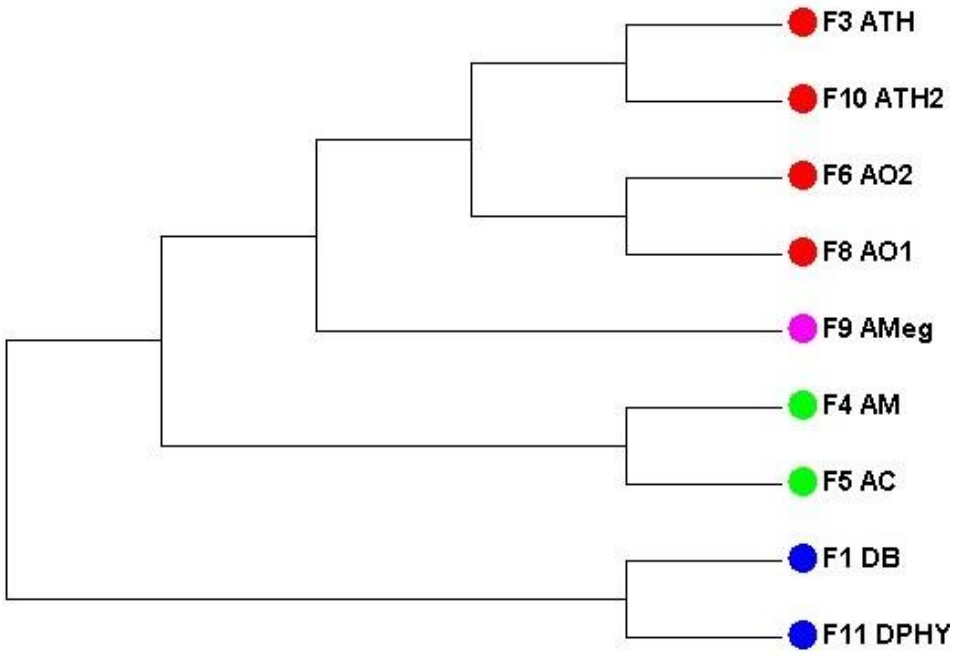


Figure 10 : trappings of *A. triticii* by several nematode trapping fungi

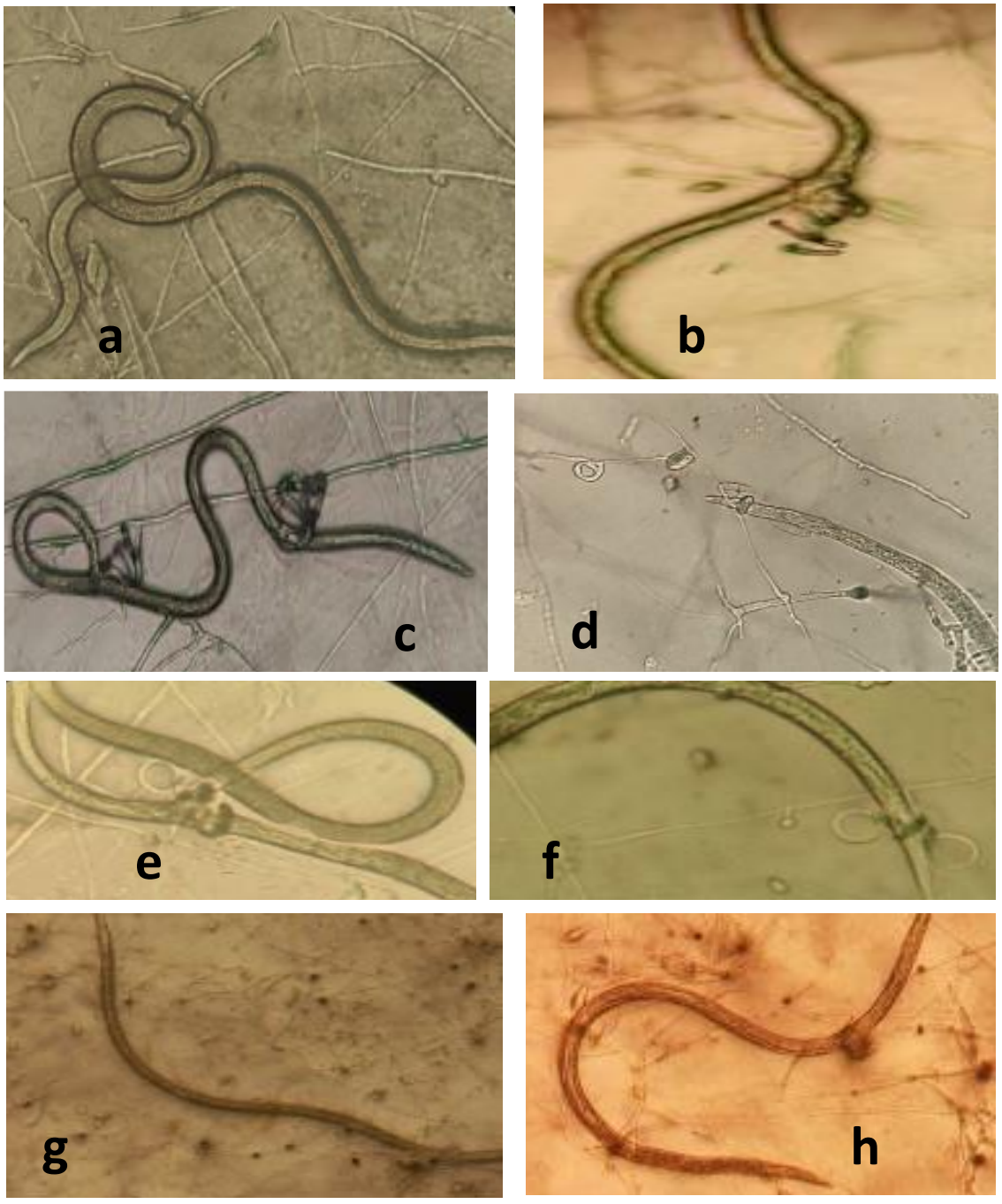


Figure: a-by ,b-by *A. conoides,* c- *A. megalospora,* d- *D. brochopaga,* e- by *A. oligospora,* f- by *A.musiformis,* g,h- by *D. phymatopaga*

Figure 11: Trapping of *M. javanica* by nematode trapping fungi

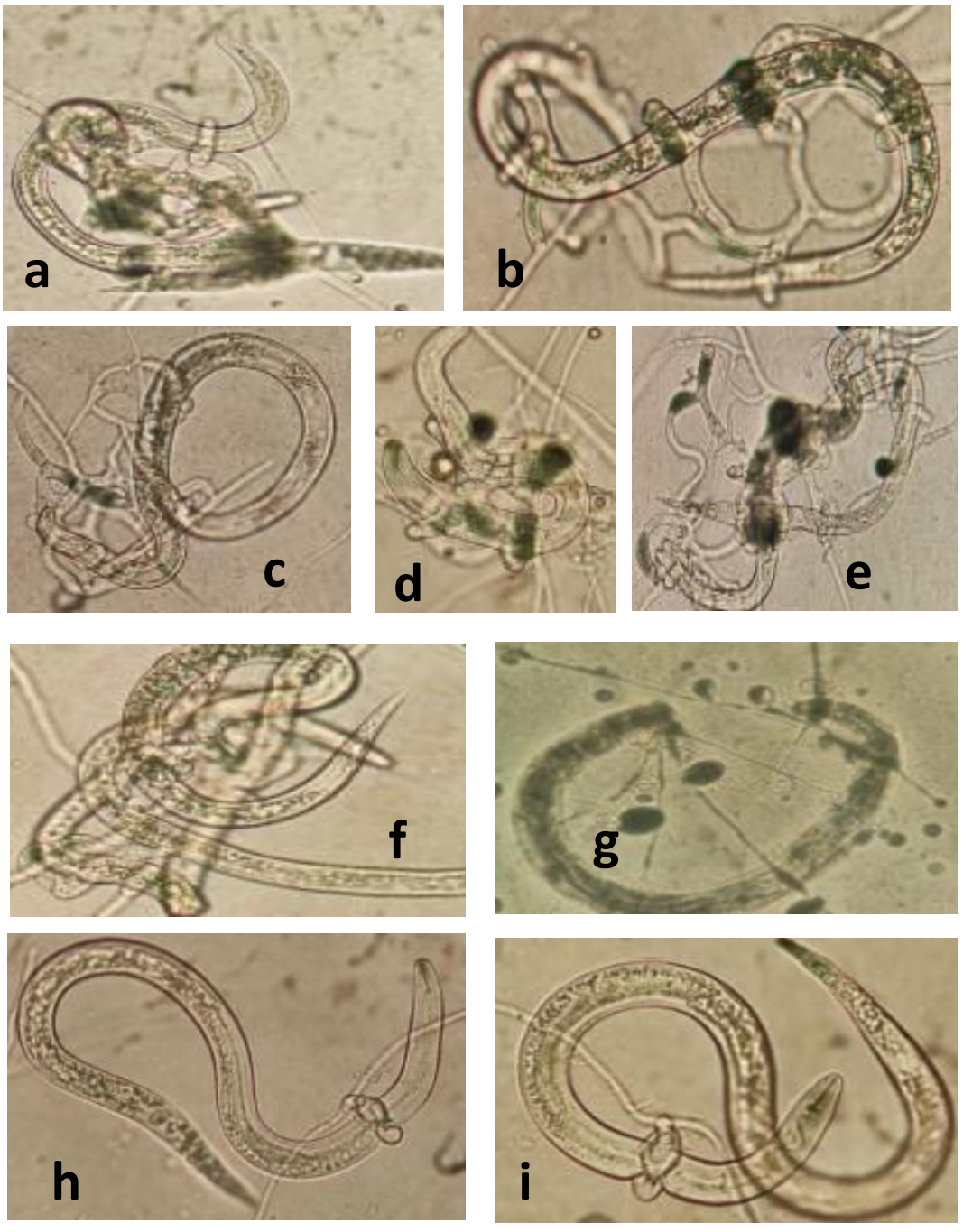


Figure: a- by *A.conoides*, b- by *A.oligospora*, c by *A. thaumasia*, d- by *A. musiformis*, e,f-by *A. megalospora*, g-h,i- by *D. brochopaga*

Figure 12: Hyphal coiling produced by nematode trapping fungi around the hyphae of *R. solani* (AG-1 & AG-3)

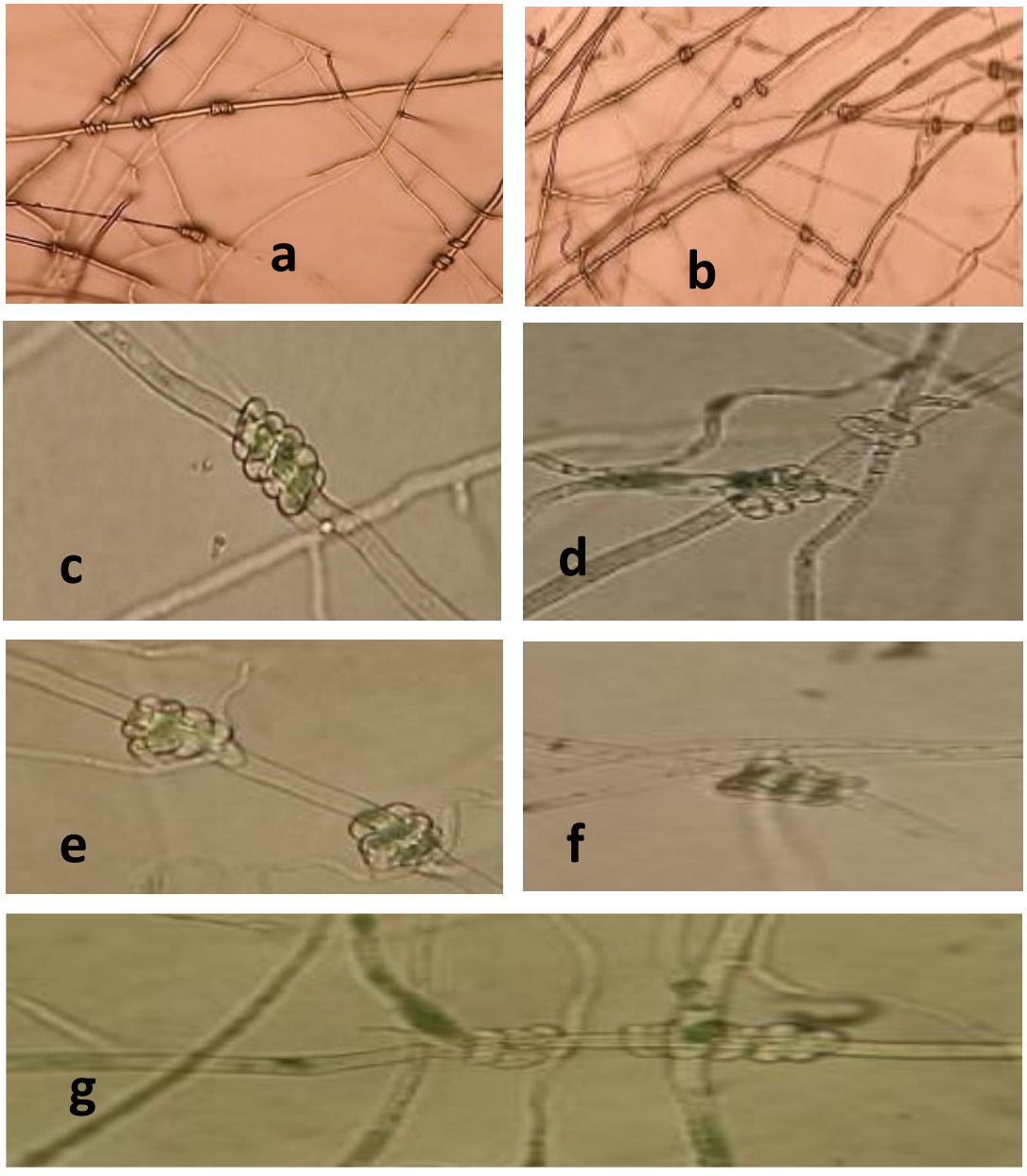


Figure: a,b observedunder 100x, c,d,e,f,g- observedunder 400x

Table 5: Similarity coefficient values among nine isolates of nematode trapping fungi based on ITS region

	1	2	3	4	5	6	7	8
A.conoides	1	2	3	4	5	6	7	8
A.musiformis	0.073							
A.oligospora 2	0.106	0.103						
A.oligospora 1	0.104	0.101	0.002					
M. sphaeroides	0.118	0.115	0.083	0.08				
A.thaumasia 2	0.112	0.102	0.065	0.063	0.099			
A.thaumasia 1	0.115	0.102	0.065	0.063	0.098	0.006		
D.brochopaga	0.268	0.28	0.27	0.267	0.265	0.256	0.263	
D.phymatopaga	0.203	0.214	0.208	0.205	0.187	0.186	0.186	0.232

Table 6: *In vitro* nematode-trapping ability of nematode trapping fungi against second juveniles of *Anguina triticii* in corn meal agar medium (0.4%)					
Nematode trapping fungi	**% trapped *A. triticii* after**				
	24h	**48h**	**72h**	**96h**	**120h**
A.conoides	9.50	21.00	29.00	37.83	49.17
A.oligospora 1	13.00	27.00	55.00	66.16	80.83
D.brochopaga	4.83	13.83	27.33	39.33	49.50
A.oligospora 2	13.33	28.83	58.17	75.83	84.33
A.musiformis	8.00	17.83	32.83	50.66	69.00
***A.thaumasia* 2**	5.67	14.33	32.67	46.66	55.67
A.thaumasia 1	7.33	16.83	37.67	49.50	60.33
M. sphaeroides	10.17	26.83	44.33	59.67	77.33
D.phymatopaga	5.50	12.33	30.67	60.83	76.00
Factors	**C.D. @ 1%**		**S.E.(d)**		**S.E.(m)**
Fungus	0.82		0.41		0.29
Time	0.61		0.31		0.22
Interaction	1.83		0.92		0.65

Line graph 1: *In vitro* nematode-trapping ability of nematode trapping fungi against second juveniles of *Anguina triticii* in corn meal agar medium (0.4%)

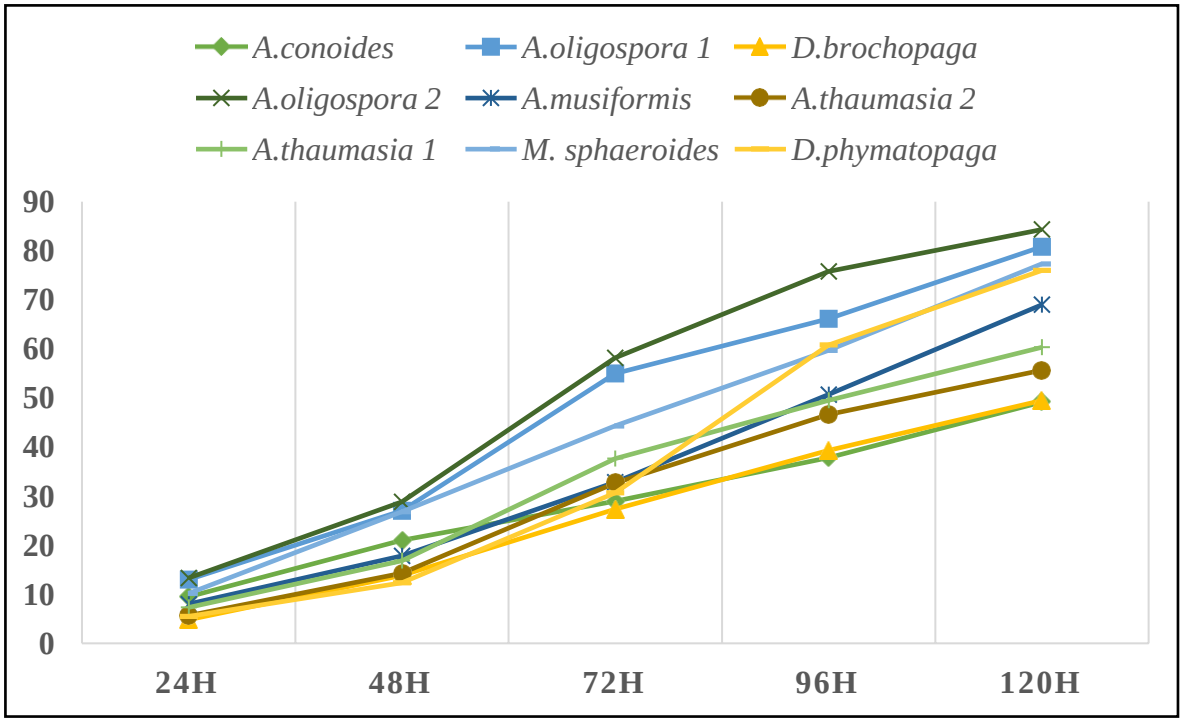


Table 7: *In vitro* trap formation in response to second stage juvenile of *A. triticii* in corn meal agar medium (0.4%)					
Nematode trapping fungi	**No. of traps formed after inoculation of 2nd juvenile of *A. triticii***				
	24h	**48h**	**72h**	**96h**	**120h**
A.conoides	0.90	2.43	7.30	10.79	12.90
A.oligospora 1	1.93	5.47	16.63	25.43	26.43
D.brochopaga	4.49	10.29	27.39	55.61	56.99
A.oligospora 2	2.53	6.53	18.45	27.67	28.58
A.musiformis	1.33	4.38	8.60	16.23	17.33
A.thaumasia 2	1.27	3.50	7.67	15.85	16.91
***A.thaumasia* 1**	1.47	3.72	8.67	17.45	18.37
M. sphaeroides	1.13	5.49	14.11	20.93	22.19
D.phymatopaga	2.50	7.03	11.50	26.20	27.52

Factors	**C.D. @ 1%**	**S.E.(d)**	**S.E.(m)**
Fungus	0.77	0.38	0.27
Time	0.58	0.29	0.21
Interaction	1.73	0.87	0.61

Line graph 2: *In vitro* trap formation in response to second stage juvenile of *A. triticii* in corn meal agar medium (0.4%)

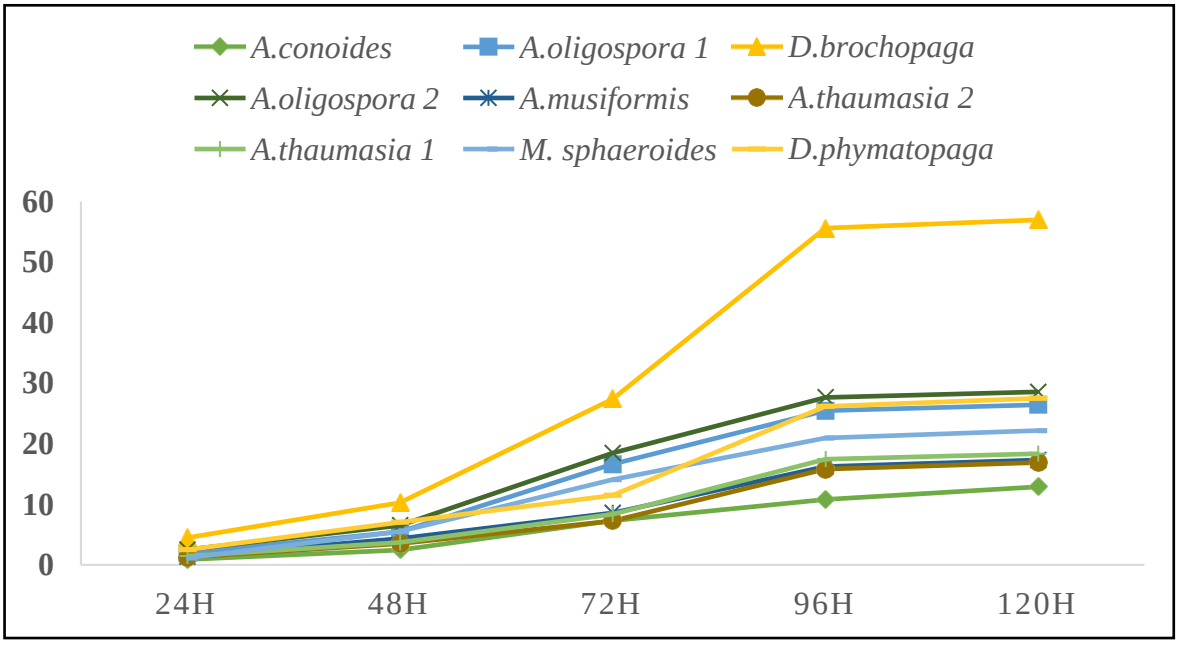


Table 8: *In vitro* nematode-trapping ability of nematode trapping fungi against second juveniles of *M. javanica* in corn meal agar medium (0.4%)					
Nematode trapping fungi	**% trapped *M. javanica* after**				
	24h	**48h**	**72h**	**96h**	**120h**
A.conoides	1.33	6.5	17.85	45.81	60.67
A.oligospora 1	3.15	12.853	30.85	61.49	72.59
D.brochopaga	9.66	23.167	52.76	75.62	92.63
A.oligospora 2	3.37	11.763	33.06	61.05	73.63
A.musiformis	1.67	7.333	18.69	47.70	65.26
A.thaumasia 2	1.83	7.433	15.99	42.72	62.32
A.thaumasia	2.07	7.167	15.54	44.61	63.72
M. sphaeroides	7.33	17.833	34.17	58.16	75.17
D.phymatopaga	7.50	18.5	35.77	61.25	79.29
Factors	**C.D. @ 1%**		**S.E.(d)**		**S.E.(m)**
Fungus	1.31		0.66		0.46
Time	0.98		0.49		0.35
Interaction	2.94		1.48		1.04

Line graph 3: *In vitro* nematode-trapping ability of nematode trapping fungi against second juveniles of *M. javanica* in corn meal agar medium (0.4%)

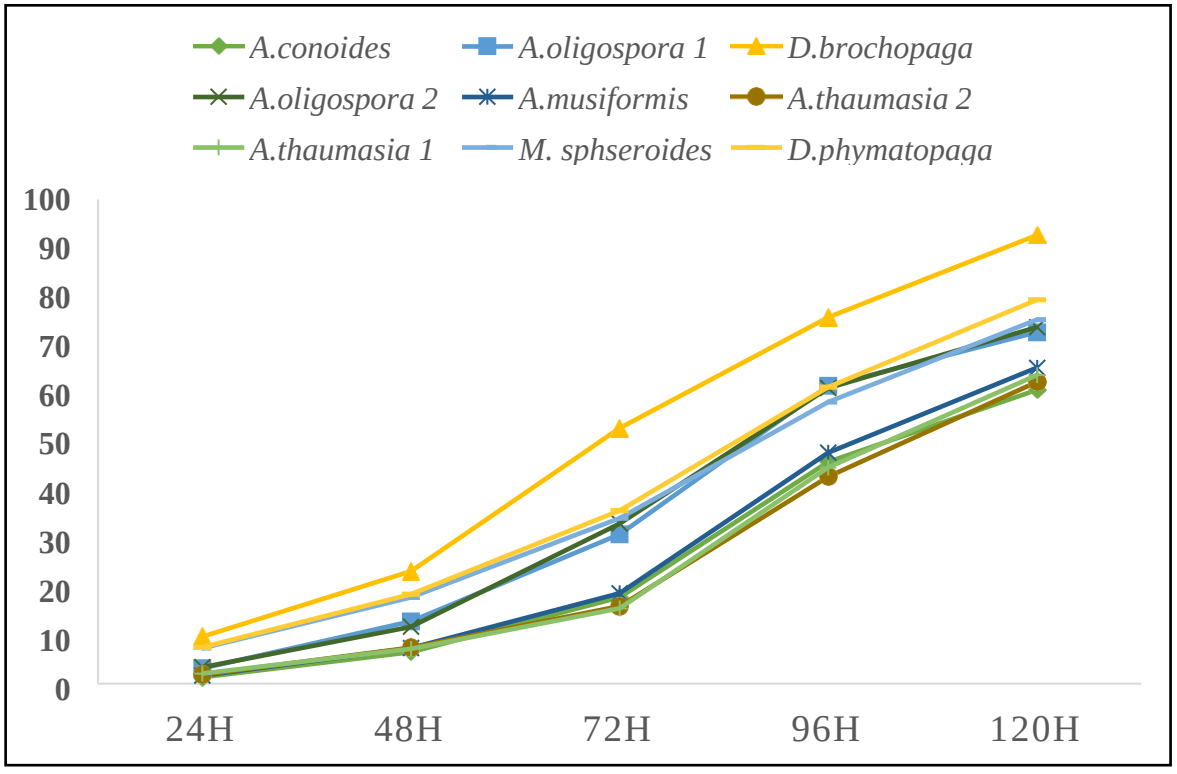


Table 9: *In vitro* trap formation in response to second stage juvenile of *M. javanica* in corn meal agar medium (0.4%)					
Nematode trapping fungi	**No. of traps formed after inoculation of 2nd juvenile of *M. javanica***				
	24h	**48h**	**72h**	**96h**	**120h**
A.conoides	0.70	2.57	6.48	9.72	12.66
A.oligospora 1	1.28	3.69	6.53	9.66	14.96
D.brochopaga	4.60	6.50	15.94	19.99	22.31
A.oligospora 2	1.17	3.59	5.55	8.55	14.63
A.musiformis	0.80	2.07	6.44	9.53	14.41
A.thaumasia 2	0.77	1.77	5.41	9.79	11.46
A.thaumasia 1	1.12	2.08	6.84	10.93	13.81
M. sphaeroides	1.13	4.05	9.63	16.23	20.09
D.phymatopaga	2.12	4.05	10.67	17.34	21.02

Factors	**C.D. @ 1%**	**S.E.(d)**	**S.E.(m)**
Fungus	0.79	0.39	0.28
Time	0.59	0.29	0.21
Interaction	1.77	0.89	0.63

Line Graph 4: *In vitro* trap formation in response to second stage juvenile of *M. javanica* in corn meal agar medium (0.4%)

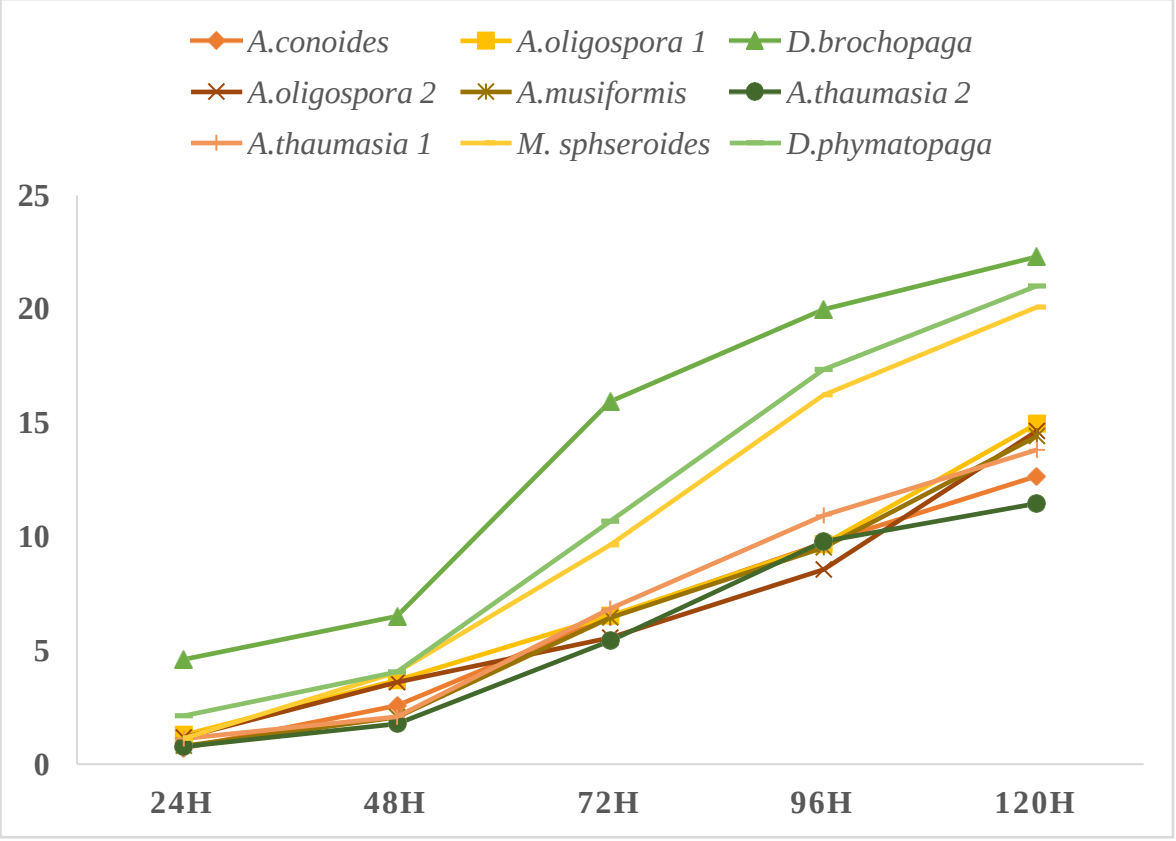
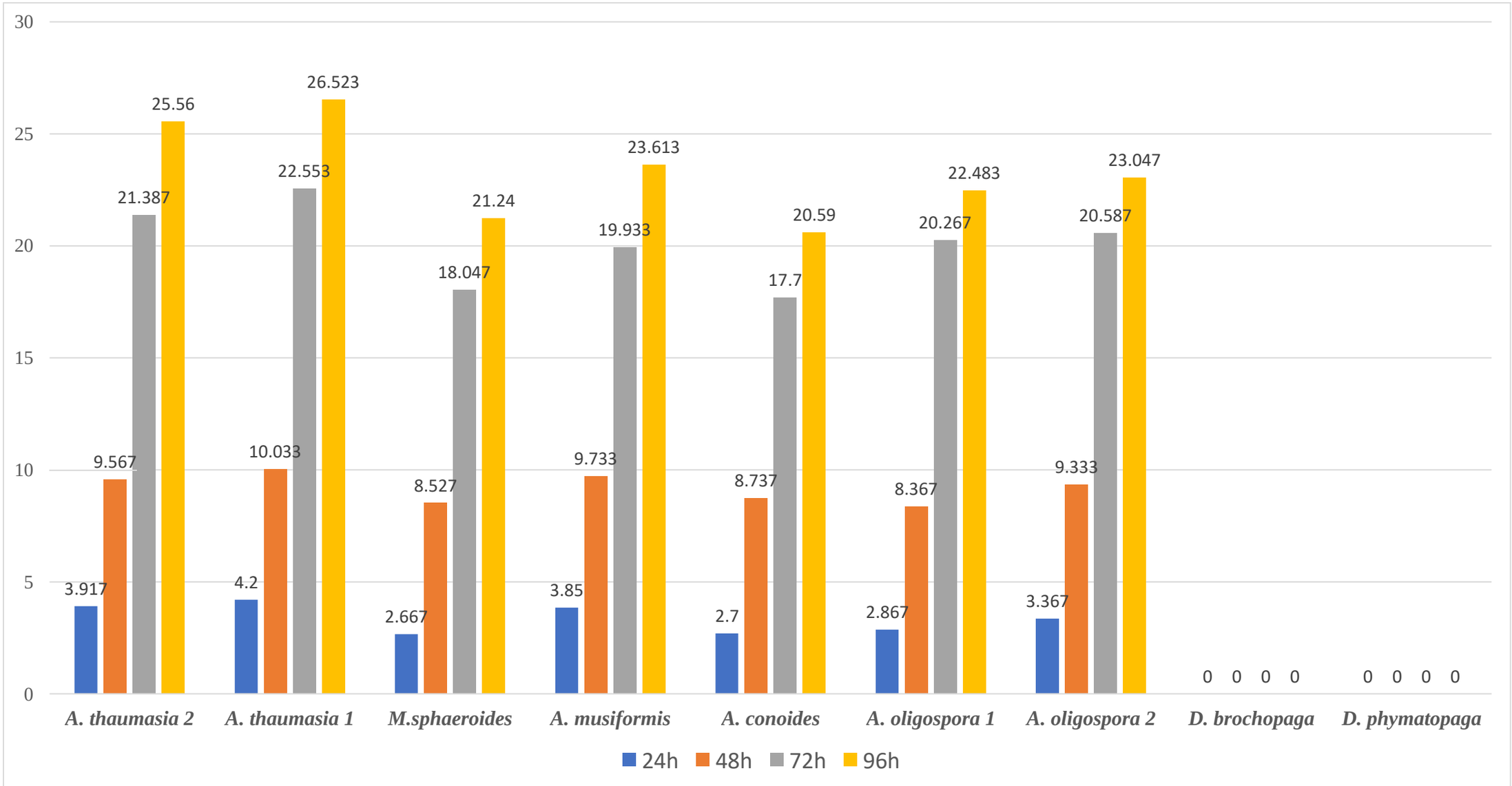


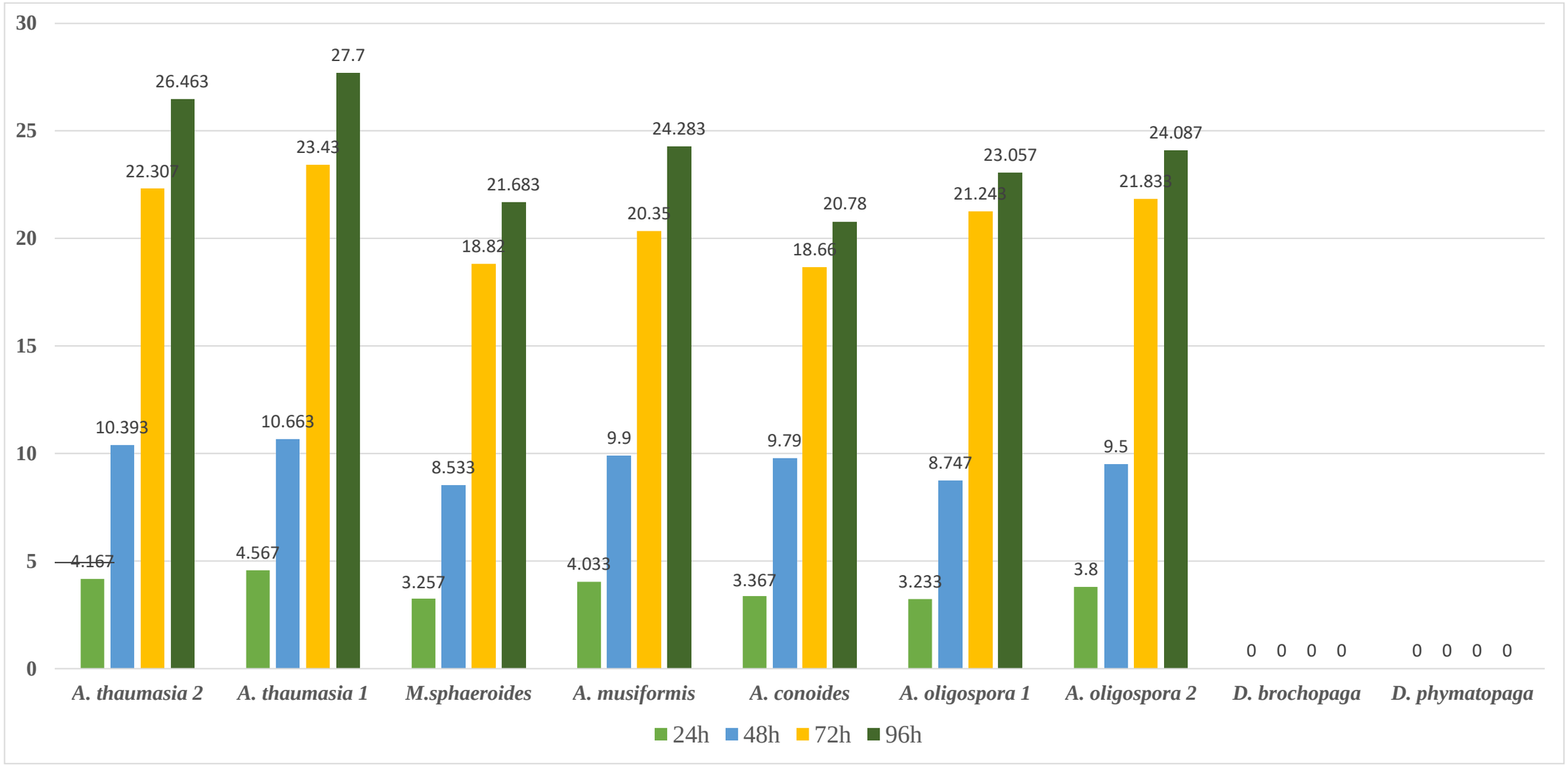
Table 10: Formation of hyphal coils around hyphae of *R. solani* (AG-1) by nematode trapping fungi during mycoparasitism				
Nematode trapping fungi	**Average number of coils after**			
	24h	**48h**	**72h**	**96h**
A. thaumasia 2	3.91	9.57	21.39	25.56
A. thaumasia 1	4.20	10.03	22.55	26.52
M. sphaeroides	2.67	8.53	18.05	21.24
A. musiformis	3.85	9.73	19.93	23.61
A. conoides	2.70	8.74	17.70	20.59
A. oligospora 1	2.87	8.37	20.27	22.48
A. oligospora 2	3.37	9.33	20.59	23.05
D. brochopaga	0	0	0	0
D. phymatopaga	0	0	0	0
Factors	**C.D. @ 1%**	**S.E.(d)**	**S.E.(m)**	
Fungus	0.92	0.46	0.32	
Time	0.69	0.35	0.24	
Interaction	1.83	0.91	0.65	

Table 11: Formation of hyphal coils around hyphae of *R. solani* (AG-3) by nematode trapping fungi during mycoparasitism				
Nematode trapping fungi	**Average number of coils after**			
	24h	**48h**	**72h**	**96h**
A. thaumasia 2	4.17	10.39	22.31	26.46
A. thaumasia 1	4.57	10.66	23.43	27.70
M. sphaeroides	3.26	8.53	18.82	21.68
A. musiformis	4.03	9.90	20.35	24.28
A. conoides	3.37	9.79	18.66	20.78
A. oligospora 1	3.23	8.75	21.24	23.06
A. oligospora 2	3.80	9.50	21.83	24.09
D. brochopaga	0	0	0	0
D. phymatopaga	0	0	0	0
Factors	**C.D. @ 1%**	**S.E.(d)**	**S.E.(m)**	
Fungus	0.89	0.44	0.31	
Time	0.67	0.33	0.24	
Interaction	1.77	0.88	0.62	

Line graph 5- Formation of hyphal coils around hyphae of Rice, *R. solani* (AG-1) by nematode trapping fungi during mycoparasitism



Line graph 6- Formation of hyphal coils around hyphae of Mungbean, *R. solani* (AG-3) by nematode trapping fungi during mycoparasitism



DISCUSSION

The occurrence of several nematode-trapping fungi namely *Drechslerella brochopaga, Arthrobotrys musiformis, Monacrosporium sphaeroides, Arthrobotrys conoides, Dactylellina phymatopaga, Arthrobotrys oligospora* isolates, and *Arthrobotrys thaumasia* isolates in culture plates plated with soil and decaying plant material of Mahoba and Banda district indicates that these fungi are abundantly found in the soil of Mahoba and Banda which are true representative of Bundelkhand, Uttar Pradesh. Wide distribution of nematode-trapping in variety of habitats such as horticultural, agricultural, and forest soil have been reported by several workers across the world (Drechsler, 1937; Duddington, 1954; Gray, 1983; Persmark and Jansson,1997; Jansson and Lopez-Llorca, 2001; Gray, 1988; Singh *et al*., 2007; Kumar and Singh, 2006). The critical morphological examination of the shape and size of conidia, their respective conidiophores and other morphological structures of nematode-trapping fungi used in current investigation were found similar to *Drechslerella brochopaga, Arthrobotrys musiformis, Monacrosporium sphaeroides, Arthrobotrys conoides, Dactylellina phymatopaga, Athrobotrys oligospora* , and *Arthrobotrys thaumasia* as reported by Dreshsler (1937) and mentioned in the identification key given by Cook and Godfrey (1964).After morphological, isolated nematode trapping fungi was molecularly characterized by sequencing the amplified PCR product and BLAST search. The amplified bands of all the isolates were observed between 600 and 750 bp (Figure-8). Hence, the nine isolates of nematode-trapping fungi studied in detail during present investigation were identified as *Drechslerella brochopaga* Drechsler, *Arthrobotrys musiformis* Drechsler, *Monacrosporium sphaeroides* Drechsler, *Arthrobotrys conoides* Drechsler, *Dectylellina phymatopaga* Drechsler, *Athrobotrys oligospora* 1 Fresen, *Athrobotrys oligospora* 2 Fresen, *Arthrobotrys thaumasia* 1 Drechsler, and *Arthrobotrys thaumasia* 2 Drechsler (Fig.1-Fig. 7). Phylogenetic analysis of all the isolatesshows that *A. thaumasia* 1 and *A. thaumasia* 2, *A. oligospora* 2 and *A. oligospora* 1 were closely related and placed in same clade respectively. *M. sphaeroides* was distantly related with others so this was placed in different clade. *A. musiformis* and *A. conoides, D. brochopaga* and *D. phymatopaga* were closely related to each other so placed in same clade respectively (Figure 9).

The abundant production of constricting rings on hyphae of *D. brochopaga,*

adhesive network in *A. musiformis,* sessile knobs in *D. phymatopaga* and three dimensional network by *A. oligospora* strains, *A. conoides, M. sphaeroides,* and *A. thaumasia* strains in response to free living nematodes during isolation suggested that three factors might have worked either alone or concomitantly such as accumulation of morphogenic substance Nemin in higher concentration, temperature, and absorption of nutrition by fungus after paralysis of nematodes body (Cruz *et al*., 2009; de Cruz *et al*., 2011). Trapping of the *A. triticii* and *M. javanica* by all the nematode trapping fungi isolates by producing adhesive trapping structures also indicates that these fungi having nematophagous ability in nature and in different habitats (Fig. 10&11).

The formation of average number of trapping structures such as constricting ring by *D. brochopaga*, Adhesive net by *A. musiformis*, sessile knobs by *D. phymatopaga* and three-dimensional adhesive network by *A. oligospora* strains, *A. thaumasia* strains, *A. conoides* and *M. sphaeroides* in 1.66 mm^2 area (10x objective lens) of hyphal growth of respective fungi after inoculation of second stage juvenile of *A. tritticii* and *M. javanica* in corn meal agar medium (0.4%) may be attributed to trap forming ability of these fungal species. Formation of abundant constricting rings by *D. brochopaga* in comparison to other fungi indicates (Table-7&9; Line Graph-2&4) the higher sensitivity of *D. brochopaga* to the trap inducing substances produced by *A. triticii* and *M. javanica* in dual culture. *A. oligospora* 2 is found to be most predacious (84.33%) followed by *A. oligospora* 1 (80.83%), *M. sphaeroides* (77.33%) and *A. conoides* is found to be less effective (49.167%) against *A.triticii* (Table-6 & Line graph 1) whereas *D. brochopaga* produces constricting ring abundantly than *A. oligospora* but is less predacious within 24 h. of interface with *A. triticii* (Table 7&Line graph 2). Higher percentage of trapping *of M. javanica* by *D. brochopaga* (92.627%) in comparison to other fungi is attributed to the higher number of traps induced within 24 h. of interface between *D. brochopaga* and *M. javanica* in comparison to interface between *M. javanica* and other fungi (Table 8&9 & Line graph 3&4). The absence of traps in nematode free culture of all nematode trapping fungal species indicated that morphogenic substance 'Nemin' released from nematodes is essential for trap formation (Roubaud and Deschiens, 1939; Pramer and Stoll, 1959). Cook (1963) and Singh *et al*. (2007) also reported that constricting ring forming fungi are more predacious than the other nematode trapping fungi forming adhesive hyphal nets. Observation of higher trapping of *Anguina triticii* by *A. oligospora* strains and *M. javanica* by *D. brochopaga* in comparison to other nematode trapping fungi indicates the possibility of the use of *A. oligospora* strains and *D. brochopaga*

as bio control agent for the management of these plant parasitic nematodes in seed gall and root knot infested fields. Two fungal isolates isolated from infected leaves of mung bean showing the symptoms of web blight and sheath blight infected rice plant was appeared as white colonywith branched mycelium at right angle, sclerotia similar to *Rhizoctonia solani* infecting mung bean and rice. Hence from these morphological characters, the isolated fungi were identified as *Rhizoctonia solani* (AG-1 & AG-3)

The variation in formation of average number of hyphal coils during mycotrophic ability of nematode - trapping fungi viz. *A. musiformis, A. conoides, A. thaumasia* strains, *M. sphaeroides* and *A. oligospora* strains to parasitize the hyphae of both the isolates of *R. solani* may be attributed to the virulence of these nematode trapping funngi and susceptibility of the *R. solani* isolates. *A. thaumasia* strains are highly mycotrophic against both the isolates of *R. solani* followed by *A. musiformis, A. oligospora* strains, *M. sphaeroides* and *A. conoides* (Table 10-11 & Line graph 5-6). The observations showed that mung bean isolates of *R. solani* (AG-3) were more susceptible to native strains of nematode trapping fungi than the Rice isolates of *R. solani.* Failure of *D. brochopaga* and *D. phymatopaga* to parasitize the any isolates of *R. solani* indicates the lack of genes of mycoparasitism in both the nematophagous fungi. Studies on phylogeny of nematode-trapping fungi also indicated that adhesive knob forming fungi are genetically different from adhesive network forming fungi (Yang *et al.*, 2007; Yang *et al.*, 2012). Hyphal coils of nematode- trapping fungi have been reported in case of several fungi (Persson *et al.*, 1985). Singh *et al.* (2012 a) also reported variable frequency of hyphal coiling by different isolates of *A. oligospora* during mycoparasitism of *R. solani* infecting rice and Tomato. It was observed that hyphae of *A. thaumasia* strains, *A. musiformis, A. oligospora* strains, *M. sphaeroides,* and *A. conoides* starts coiling within 24 hours ofhyphal interface between mycoparasite and test pathogen. No any hyphal coiling was observed under the light microscope by *D. phymatopaga* and *D. brochopaga*. However, tested nematode-trapping fungi caused a strong proliferation of the cell wall of the *R. solani* hyphal cells. The interaction between mycotrophic nematode-trapping fungi and *R. solani* isolates is interpreted in term of competition for nutrients (Persson *et al.*, 1985) and cell apposition of papillae – like deposition in mycoparasitic system (Tzean and Estey, 1978). Observation of collapse of coiled hyphae of *R. solani* by hyphal coils of nematode- trapping fungi during mycoparasitism indicates the possibility of the use of these fungi as substitute of chemical fungicides.

SUMMARY AND CONCLUSION

*Meloidogyne javanica,*and *Anguina triticii* are the most infectious phytopathogenic nematodes that cause root- knot disease in many agricultural and horticultural crops and ear cockle disease of wheat. Currently the management of these infectious nematodes are totally relied on the use of synthetic nematicides which are highly hazardous to environment and human and leads to very noxious residual effect on non-targeted organism and vegetation. So, the development of an alternative, ecofriendly, safe and effective approaches to manage these phytopathogenic nematodes is need of the current time. *Rhizctonia solani* (Teleomorph: *Thanetophorus cucumeris*) is a widely distributed, soil borne fungal plant pathogen having a necrotrophic life style to incite the sheath blight disease of rice (*Oryza sativa* L.) and web blight of mung bean (*Vigna radiata* L.). Both the diseases cause heavy reduction in yield in all rice and mung bean growing regions of the world. Two isolates of *Rhizoctonia solani* were isolated from infected plant part of rice andmung bean as the test pathogen to explore mycoparasitic efficacy of these fungi. So, the current investigation was totally depended on to explore the biocontrol efficacy of nematode trapping fungi, that have ability to capture, kill and digest these infectiousphytopathogenic nematodes completely.

During the research work, nine nematode trapping fungal spp. were isolated from different locations of two district (Banda and Mahoba) of Bundelkhand region of Uttar Pradesh. These nematode trapping fungi namely *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys thaumasia 1, Arthrobotrys thaumasia 2, Arthrobotrys conoides, Dactylellina phymatopaga, Monacrosporium sphaeroides, Arthrobotrys oligospora 1 and Arthrobotrys oligospora 2* were isolated and identified based on relevant literature and molecularly characterized based on BLAST search and compared with NCBI database. Presence of these fungal biocontrol agent in Bundelkhand region is blessing for thefarmers that naturally suppress these phytopathogens. All the nematode trapping fungi produce special trapping structure to capture and kill nematodes were studied in detail withspecial emphasis on their *in vitro* nematophagous and mycoparasitic ability.

In vitro nematophagous potential of these fungi was studied by inoculation of 200 juveniles of *M. javanica* and *A. triticii* in pure culture in CMA medium (0.4%). Trap formation was observed within 24 h. of nematode inoculation in all the fungi, however, the

induction of number of trapping structures of *D. brochopaga* was greater than the other fungi in 1.66 mm^2 area of fungal hyphae. The average number of traps and percent capturing of nematodes increased with the passage of time. *A. oligospora* strains were found to be most effective (84.33%) against *A. triticii* but the number of trap formation was highest (56.99) in *D. brochopaga* whereas *D. brochopaga* was found to be most predacious (92.62%) and also produce maximum number trapping structures (22.30) against *M. javanica* within 120 hours of interaction between fungus and nematodes. *A. conoides* was less predacious against both nematodes and produced lowest number of tapping structures (12.9) against *A. triticii* within 120 hours of interaction with nematodes.

During search for mycoparasitic biocontrol agent, nine *spp.*/strains tested against *R solani* (AG-1& AG-3), seven strains were found mycoparasitic against both the isolates of *R. solani* (AG-1&AG-3) *invitro.* Seven isolates *viz. Arthrobotrys musiformis, Arthrobotrys thaumasia 1, Arthrobotrys thaumasia 2, Arthrobotrys conoides, Monacrosporium sphaeroides, Arthrobotrys oligospora 1 and Arthrobotrys oligospora 2* were found mycoparasitic on hyphae of *R. solani* by forming hyphal coils around the hyphae. *Dactylellina phymatopaga* and *D. brochopaga* were failed to parasitize the hyphae of any isolate of *R. solani* which indicates that these fungi are not mycophagous. In dual cultures, the interaction between *Arthrobotrys musiformis, Arthrobotrys thaumasia 1, Arthrobotrys thaumasia 2, Arthrobotrys conoides, Monacrosporium sphaeroides, Arthrobotrys oligospora* 1 *and Arthrobotrys oligospora* 2 and isolates of *Rhizoctonia solani* occurred within 24 hours of hyphal interface on dialysis membrane. Hyphal coiling was observed around the hyphae of *R. solani* in variable frequency depending on the isolates of pathogen and species of nematode-trapping fungi. The hyphal coiling increased with the course of time. Maximum hyphal coiling was observed during interaction of *R. solani* isolates with *A.thaumasia* isolates. *D. brochopaga* and *D. phymatopaga* were failed to parasitize the hyphae of *R. solani* (AG-1 & AG-3). *A. thaumasia* strains was found most mycoparasitic on both the isolates of *R. solani* followed by *A. musiformis, A. oligospora* isolates, *M. sphaeroides* and *A. conoides.* At the site of coiling, a strong cell wall proliferation was observed in the *Rhizoctonia* cells. The cytoplasm of *R. solani* cells subsequently disintegrated which resulted in collapse of the hyphae.

On the basis of current research entitled "Exploration of nematophagous and mycoparasitc potential of native strains of nematode trapping fungi of Bundelkhand region against some plant pathogens" the following conclusions were made:

- Presence of *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys thaumasia 1, Arthrobotrys thaumasia 2, Arthrobotrys conoides, Dectylellina phymatopaga, Monacrosporium sphaeroides, Arthrobotrys oligospora 1 and Arthrobotrys oligospora 2* in soil of Banda district indicates that nematode-trapping fungi are essential component of the soil of Bundelkhand region of Uttar Pradesh.
- *A.oligospora* Strains were highly nematophagous against *A. triticii* as compared to other nematode trapping fungi due to abundantly formation of their three-dimensional trapping structure which is best fitted for capturing large sized nematode *A. triticii*.
- *D. brochopaga* was found most efficient trapper of *M. javanica* due to abundantly formation of constricting ring and killing of *M. javanica* which is best fitted for their size of constricting ring.
- Due to high trapp formation and trapping of *M. javanica, D. brochopaga* and *D. phymatopaga* could be used as potential bioagent in root knot infested soil
- Nematode-trapping fungi studied during this investigation are potent mycoparasite of *R. solani* isolates except *D. phymatopaga* and *D.brochopaga.* The effective coiling of nematode-trapping fungi around the hyphae of *R. solani* could be harnessed for their potential use in the integrated disease management programme against both nematodes and *R. solani.*

LITERATURE CITED

Ali, A. H. H. 1990. The use of nematodes trapping fungi and organic amendments to control root-knot nematodes. *Bulletin of Faculty of Agriculture*, University of Cairo, 41 (3): Suppl, 1:1001-1012.

Anderson NA 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology*; 20:329–347.

Anwar, S.A., Zia, A., Hussain, M. and Kamran, M., 2007. Host suitability of selected plants to *Meloidogyne incognita* in the Punjab, Pakistan. *International Journal of Nematology*, *17*(2), p.144.

Arndt, M., Leuprecht, B. 1994. Trials on alternatives for control of nematodes in vegetable crops. *Gartenbau-Magazine*, 3 (5): 24-25.

Balan, J. and Lechevalier, H. A., 1972. The predacious fungus *Arthrobotrys dactyloides*: induction of trap formation. *Mycologia*, 64: 919-922.

Barron, G. L., 1969. Isolation and maintenance of endoparasitic nematophagous Hyphomycetes. *Canadian Journal of Botany*, 47: 1899-1902.

Barron, G. L., 1977. The nematode- destroying fungi. In tropics in Mycobiology No. 1 (Guelph, ON, Canada: *Canadian Biological Publications Ltd.*)

Bartnicki-Garcia, S., Eren, J. and Pramer, D. 1964. Carbon dioxide dependent morphogenesis in *Arthrobotrys conoides*. *Nature*, 204: 804.

Belder, D., Efje. 1994. Biological control of plant parasitic nematodes using nematophagousfungi. Pub. No.: WO/1994/024871.

Belder, E den, Jansen, E., 1994. The influence of temperature, nutrition, light and the growth time of the mycelium on capture and infection of *Meloidogyne hapla* and *Arthrobotrys oligospora*. *Fundamental and Applied Nematology*, 17 (1): 56-66.

Butler, E.E. 1957. *Rhizoctonia solani* as a parasite of fungi. Mycologia 54,72-77.

Caryol, J. C. and. Sawadogo, A. 1991. A new African isolate of *Monacrosporium bembicoides*acting simultaneously as predator and hatching inhibitor of nematodes. *Revue- de- Nematologie*, 14 (2): 322-325.

Castillo, M.B., Alejar, M.S. and Litsinger, J.A. 1977. Pathological reactions and yield loss of mungbean to known populations of *Rotylenchulus reniformis* and *Meloidogyne acrita. Philippine Agriculturist*, 61: 12–24.

Chahal K. S., Sokhi S. S., Rattan G. S. 2003. Investigations on sheath blight of rice inPunjab. *Indian Phytopathol.* 56 22–26.

Chauhan, J. B., Priya, R. S., Jarullah, B., & Subramanian, R. B. 2007. Isolation and molecular characterization of β-tubulin gene from Arthrobotrys musiformis—A nematode trapping fungus.

Chet, I., Inbar J., Hadar I.,1997. Fungal antagonists and mycoparasites. In: Wicklow, D.T., Soderstrom, B. (Eds.), The Mycota IV: Environmental and microbial relationships. Springer-Verlag, Berlin. pp. 165-184.

Colombo, A., Sotino, O., Cosentino, S., Nucifora, A., 1995. Application of predatory fungi forthe control of root knot nematodes on eggplant in an unheated plastic house. *Nematologica Mediterranea*, 23: Suppl, 149-154.

Consolo, V.F., Monaco, C.I., Cordo, C.A., Salemo, G.L. 2012. Characterization of noval *Trichoderma* spp. isolates as a search for effective biocontrollers of fungal diseases of economically important crops in Argentina. World J. Microbiol. Biotech. 28, 1389- 1398.

Cooke, R. C. 1962. The ecology of nematode trapping fungi in the soil. *Annals of Applied Biology,* 50:507-513.

Cooke, R. C. 1962b. The behaviour of nematode- trapping fungi during the decomposition of organic matter in the soil. *Trans. Br. Mycol. Soc*., 45: 314-319.

Cooke, R. C. 1963a. Ecological characteristics of nematode-trapping Hyphomycetes. I. Preliminary studies. *Annals of Applied Biology*, 52: 431-437.

Cooke, R. C. 1969. Two nematode trapping hyphomycetes. *Duddingtonia flagrans* gen et comb. nov. and *Monacrosporium mutabilis* sp. nov. *Trans. Br. Mycol. Soc*., 53: 315- 318.

Cooke, R. C. 1977. The biology of symbiotic fungi. *John Willy and Sons, London*.

Cooke, R. C. and Dickinson, C. H. 1965. Nematode trapping species of *Dactylella and Monacrosporium*. *Trans. Br. Mycol. Soc.*, 48(4): 621-629.

Cooke, R. C. and Pramer, D. 1968. *Monacrosporium rutgeriensis* sp.n., a nematode trappingHyphomycete. *Phytopath.*, 58: 544-545.

Cooke, R. C., Godfrey BES. 1964. A key to the nematode destroying fungi. *Trans. Br. Mycol. Soc.*, 47(1):61-74.

Cooke, R.C., 1964. Ecological characteristics of nematode-trapping Hyphomycetes: II. Germination of conidia in soil. *Annals of Applied Biology*, *54*(3), pp.375-379.

Cruz, D.G., Carneiro, C.N.B., Silva, C.P., Retamal, C.A., Thiébaut, J.T.L., DaMatta, R.A., Santos, C.P., 2009. Acid phosphatase activity during the interaction of the nematophagous fungus *Duddingtonia flagrans* with the nematode *Panagrellus* sp. *Journal of Invertebrate Pathology* 102, 238–244.

Dackman, C., Nordbring-Hertz, B. 1992. Conidial traps – a new survival structure of the nematode-trapping fungus *Arthrobotrys oligospora*. *Mycolical Research*, 96*:* 194–198.

Dayal, R, Gupta RN. 1975b. Studies in predacious fungi IV. Some members of Hyphomycetes. *Proceedings of National Academy of Science India*; 45: 237-240.

Dayal, R., Nand, R. 1973a. Studies on predacious fungi I. Zoopagales. *Proc. National Academy of Science India*, 43: 87-92.

Dayal, R., Nand, R. 1973b. Studies on predacious fungi II. Some members of Hyphomycetes. *Proc. National Academy of Science India*, 43:249-254.

Dayal, R., Singh, V. K. 1975. Studies on predacious fungi III. Some members of Hyphomycetes. *Proceedings of National Academy of Science India*, 45:89-92.

Dayal, R., Srivastava, S. S. 1978. Studies on predacious fungi V. Some members of Hyphomycetes. *Proceedings of National Academy of Science India*, 48: 88-92.

de Cruz, D.G., Araujo, F.B., Molento, M.B., DaMatta, R.A., Santos, C.P., 2011. Kineticsof capture and infection of infective larvae of trichostrongylides and free-living nematodes *Panagrellus sp.* by *Duddingtonia flagrans.* Parasitology Research (doi:10.1007/s00436-011-2350-3).

De Gives, M. P., Zavaleta, M. E., Herrera- Rodriguez, D. and Quiroz, R. H. 1994. *In vitro* trapping capability of *Arthrobotrys* spp. on infective larvae of *Haemonchus contortus* and *Nacobbus aberrans*. *Journal of Helminthology*, 68 (3): 223-229.

Dobbs, C. G., Hinson, W. H. 1953. A wide spread fungistasis in soil. *Nature*, 172: 197-199.

Drechsler, C. 1933. Morphological diversity among fungi capturing and destroying nematodes. *Journal of Washington Acadamic Sci*ence, 23: 138-141.

Drechsler, C. 1933a. Morphological features of some more fungi that capture and kill nematodes. *Journal of Washington Acadamic Sci*ence, 23:267-270.

Drechsler, C. 1937. Some Hyphomycetes that prey on free living terricolous nematodes. *Mycologia*, 29: 447-552.

Drechsler, C. 1941. Predaceous fungi. *Biological Reviews*, *16*(4), pp.265-290.

Duddington, C. L. 1957. The friendly fungi: A new approach to the eelworm problem. *Faberand Faber, London.*

Duddington, C. L. 1962. Predacious fungi and control of eelworms, *In view points in Biology,Vol.1* (C. L. Duddington and J. D. Carthy, Eds.). Butterworths. London.

Dwivedi RP, Saksena HK 1974. Occurance of web blight caused by *Thanatephorus cucumeris* on mung bean. Indian journal farm science; 2:100.

Esser, R. P., Gholl, N. E. and Price, M. 1991. Biology of *Dactylella megalospora* Drechs, a nematophogous fungus. *Proceedings of soil and Crop Science Society of Florida*, 173- 180.

Exner B and Chilton SJB., 1943. Cultural differences among single basidiospore isolate of *Rhizoctonia solani*. *Phytopathology*; 33:171-174.

Falbo, M. K., Soccol, V. T., Sandini, I. E., Vicente, V. A., Robl, D., & Soccol, C. R., 2013. Isolation and characterization of the nematophagous fungus *Arthrobotrys conoides*. *Parasitology research*, *112*, 177-185.

Feder W A, Everard C O R, Duddington C L., 1960. Heterocaryotic nature of ring formation in predacious fungus *Dactylella doedycoides.* *Science*; 131: 922-924.

Feder WA, Everard COR, Wotton LMO., 1963. Sensitivity of several nematophagous fungus *Dactylella* to a morphogenic substance derived to free-living nematode. *Nematologica*; 9: 49-54.

Fresnius, G. 1852. *Beitrage Zur Mykologie*. Heft, 1-2 pp. 1-80.

Galper, S., Eden, L. M., Stirling, G. R., Smith, L. J. 1995 Simple methods of assessing thepredacious activity of Nematode- trapping fungi. *Nematologica* 41, 130-140.

Gonzalez GV, Portal Onco MA, Rubio SV. 2006. Review. Biology and systematics of theform genus *Rhizoctonia*. *Spain Journal of Agricultural Research*; 4:55–79.

Gray, N. F. 1983b. Ecology of nematophagous fungi: *Panagrellus redivivus* as the target organism. *Plant and Soil*, 73: 293-297.

Gray, N. F., 1983. Ecology of nematophagous fungi: distribution and habitat. *Annals of Applied Biology*, *102*(3), pp.501-509.

Guillemaut C, Edel-Hermann V, Camporota P, Alabouvette C, Richard-Molard M, Steinberg C. 2003. Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis ofribosomal DNA. *Canada Journal of Microbiology*; 49:556–568.

Gui-Zhen, Ma, Shi-dong, Li, Bing-Yan, Xie, Guo-Zheng, Lu, 2004. Mycoprasitism of nematode- trapping fungus *Monacrosporium ellipsosporum* and its biochemical basis. Agri. Si. China 3 (1)**,** 37-43.

Gupta, D., Shome, S. N., Usha Shome, S. K. 1964. Preliminary report of predacious fungi in India. *Current Science,* 33:380-381.

Heintz, E. C. 1978. Assessing the predacity of nematode trapping fungi *in vitro*. *Mycologia*, 70: 1086-1100.

Jain, R.K., Mathur, K.N. and Singh, R.V. 2007. Estimation of losses due to plant parasitic nematodes on different crops in India. *Indian Journal of Nematology.* 37: 23-25.

Jansson, H. B. and Nordbring-Hertz, B. 1980. Interactions between nematophagous fungi and plant-parasitic nematodes: attraction, induction of trap formation and capture. *Nematologica*, 26: 383-389.

Juniper, A. J. 1957. Dung as a source of predaceous fungi. *Transactions of British Mycological Society,* 40: 346-348.

Keiser, A., Häberli, M. and Stamp, P. 2012. Quality deficiencies on potato (*Solanum tuberosum* L.) tubers caused by *Rhizoctonia solani*, wireworms (*Agriotes*sp.) and slugs(*Deroceras reticulatum*) in different farming systems. *Field Crops Research*, 128: 147-155.

Kerry, B. 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annual Review of Phytopatholgy* 38, 423–41.

Khushbu, S., 2016. Effect of bio-agents as soil application against root-knot nematode, *Meloidogyne incognita* infecting brinjal. *International Journal of Agriculture Sciences,ISSN*, pp.0975-3710.

Kohli CK. 1966. Pathogenicity and host range studies on paddy sheath blight pathogen *Rhizoctonia solani*. *Journal and Research, P.A.U.* 3:7-40.

Krishanppa, K. and Shreenivasa, K.R. 2009. Biological control of Plant Parasitic Nematodes. *Agrotech Publishing Academy, Udaipur*. pp 54.

Kumar, D. 2003. Studies on predacity and biocontrol potential of *Arthrobotrys dactyloides*. Ph.D. *Thesis, Banaras Hindu University, Varanasi, India.*

Kumar, D., Maurya, N., Kumar, P., Singh, H., Addy, S.K., 2015. Assessment of germination and carnivorous activities of a nematode-trapping fungus *Arthrobotrys dactyloides* in fungistatic and fungicidal soil environment. Biol. Control 82, 76-85.

Kumar, D., Singh, K. P. 2006. Assessment of predacity and effect of *Arthrobotrys dactyloides* for biological control of root knot disease of tomato. *Journal of Phytopathology*154, 1–5.

Kumar, N., Singh, K. P. 2011. Bioefficacy of Spore Suspensions and Mass Culture of *Dactylaria brochopaga* on *Meloidogyne incognita* (Kofoid and White) Chitwood Causing Root-Knot Disease of Tomato (*Lycopersicon esculentum* Mill.). *Asian Journalof Plant Pathology, 5: 54-61*.

Kumar,K., Amaresan, N., Bhagar, S., Madhuri, K., Srivastava, R.C., 2012. Isolation and characterization of *Trhichoderma* spp. for antagonisitic activity against root rot and foliar pathogens. *Indian J. Microbiol*. 52, 133-144.

Kumar,K., Amaresan, N., Bhagar, S., Madhuri, K., Updhayaraj P., Srivastava, R.C., 2011. Genetic and physiological relatedness of antagonistic *Trichoderma* isolates against soil borne plant pathogenic fungi. *Arch Phytopathol*. Pl. Prot. 44, 1399-1409.

Larsen, M., 2000. Prospects for controlling animal parasitic nematodes by predacious micro fungi. *Parasitology*, vol. 120, no. 7, suppl., pp. 121-131.

Lee FN and Rush MC., 1983. Rice sheath blight: a major rice disease. *Plant Diseases*; 67:829–832

Leukel, R.W., 1957. *Nematode Disease of wheat and rye*. USDA Farmers Bulletin, 1607. Linford, M. B. 1937. Stimulated activity of natural enemies of nematodes. *Science*, 85: 123-124.

Linford, M. B. and Yap, F. 1939. Root knot nematode injury restricted by a fungus. *Phytopatology*, 29: 596-609.

Linford, M. B., Yap, F. 1938. Root knot injury restricted by a nematode trapping fungus. *Phytopathology*, 28: 14-15.

Lowton, J. R. 1957. The formation of traps in nematode catching Hyphomycetes grown in pure culture. *J. Expt. Bot.*, 8: 50-57

Mankau, R. 1962. Soil fungistasis and nematophagous fungi. *Phytopathology,* 52 (7): 611615. Mankau, R. 1980. Bio- control: Fungi as nematode control agent. *Journal of Nematology*, 12(4): 244-252.

Mankau, R., Clark, O. F. 1959. Nematode-trapping fungi in southern California citrus soil. *Plant Disease Reporter*, 43: 968-969.

Maqbool, M.A., 1988. In: *Nematodes parasitic to cereals and legumes in temperate semi-arid regions* (eds. M.C. Saxena, R.A. Sikora and J.P. Srivastava). ICARDA,

Aleppo, Syria, pp. 173-180.

Margani R, Widadi S. 2018. Utilizing Bacillus to inhibit the growth and infection by sheath blight pathogen, *Rhizoctonia solani* in rice. IOP conference series: earth and environmental science, Vol. 142, No. 1. IOP Publishing, Bristol

Matsumoto T and Yamoto E. 1935. *Hypochnus sasakii* Shirai in comparision with *Corticium stevensii* Brut. And *Corticium coleroga*(Cook) V. Hohn. Trans *National Histological Society*; 25:161-175.

Monoson, H. L. 1968. Trapping effectiveness of five species of nematophagous fungi culturedwith mycophagous nematodes. *Mycologia,* 60: 788-801.

Monsoon H L, Galsky A G and Stephano RS. 1974. Studies on ability of various nematodes to induce trap formation in Nematode-trapping fungus *Monacrosporium doedycoies*. *Nematologica*; 20: 96-102.

Monsoon, H. L., Ranieri, GM. 1972. Nematode attraction by an extract of a predaceous fungus. *Mycologia,* 64: 628-631.

Nacien CC 1924. Studied on *Rhizoctonia* blight of beans. Philippine Agriculturist.;8:315-321.

Nordbring-Hertz, B. 1973. Peptide induced morphogenesis in the nematode trappingfungus *Arthrobotrys oligospora*. *Physiol. Plant*., 29: 279-284.

Nordbring-Hertz, B., Jansson, H. B., Tunlid, A. 2006. Nematophagous fungi. In Encyclopediaof life sciences. *Chichester: John Wiley & Sons, Ltd.*

Ocampo-Gutiérrez, A. Y., Hernández-Velázquez, V. M., Aguilar-Marcelino, L., Cardoso-Taketa, A., Zamilpa, A., López-Arellano, M. E., Mendoza-de Gives, P. 2021. Morphological and molecular characterization, predatory behaviour and effect of organic extracts of four nematophagous fungi from Mexico. *Fungal Ecology*, *49*, 101004.

Ogbuji, R.O. 2004. Soil depth distribution of the root-knot nematode (*Meloidogyne incognita*) from two farmlands in a humid tropical environment. *Geo Journal* 5, 79-80.

Ogoshi A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. *Annual Review of Phytopathology*; 25:125–143.

Palo MA. 1926. *Rhizoctonia* diseases of rice I.A. study of the disease and of the influence of certain conditions upon viability of the scretioal bodies of the causal fungus. *PhilippineAgriculture*; 15:361-376.

Pan XB, Rush MC, Sha XY, Xie QJ, Liscombe SD, Stenina SR, Orad JH. 1999. Major gene non-allelic sheath blight resistance from the rice cultivars Jasmine 85 and Teqing. *CropScience*; 39:338–346.

Pandit, R. J., Kunjadia, P. D., Mukhopadhyaya, P. N., Joshi, C. G., & Nagee, A. H. 2014. Isolation, molecular characterization and predatory activity of two Indian isolates of nematode-trapping fungi. *Applied Biological Research, 16*(1), 10-20.

Papavizas, G.C., 1985. Trichoderma and Gliocladium: Biology, ecology and potential for biocontrol. *Annu. Rev. Phytopathol*. 23, 23-54.

Paracer CS and Chahal DS. 1963. Sheath blight of rice caused by *Rhizoctonia solani*- A new record in India. *Current Science*; 32:328-329.

Paruthi, I.J. and Bhatti, D.S., 1988. *Haryana Agric. Uni. J. Res*., **18**: 173-176.Paruthi, I.J. and Gupta, D.C., 1987. *Harayana Agric. Uni. J. Res*., **17**: 78-79. Paruthi, I.J., Singh, M. and Gupta, D.C., 1987. *Seed Res*., **15**: 83-86.

Patil, S. D., Pendse, M. A. 1976. Some endozoic nematophagous fungi from Maharashtra –India. *M.V.M. Patrika*, 11: 2730.

Patil, S. D., Pendse, M. A. 1981. Ecological studies of nematophagous and zoophagous fungiin Maharashtra. *Journal University of Poona, Science and Technology,* 54: 197-201.

Persmark, L., Nordbring- Hertz, B. 1997. Conidial trap formation of nematode-trapping fungiin soil and soil extracts. *FEMS-Microbiology-Ecology,* 22: 4, 313-323.

Person Y and Bååth E. 1992. Quantification of mycoparasitism by the nematode-trapping fungus *Arthrobotrys oligospora* ann *Rhizoctonia solani* and the influence of nutrient levels. *FEMS Microbiology Letters*; 101:11-16.

Persson Y, Veenhuis M and Nordbring-Hertz B. 1985. Morphogenesis and significance of hyphal coiling by nematode-trapping fungi in mycoparasitic relationships. In: *FEMS Microbial Ecology*; 31(5): 283-291.

Pramer D and Stoll NR. 1959. Nemin : a morphogenic substance causing trap formation by predacious fungi. *Science,* 129: 966-967.

Pramer, D. 1964. Nematode- trapping fungi. *Science* 144:382-388.

Prasad, G. 1985. Studies on nematophagous fungi. Ph.D. Thesis. *Bananas Hindu University.*

Prasad, G., Singh P.R. Dayal, R.,1984a. Studies on nematophagous fungi VI: Two new records from India. *National Academy of Science Letters*; 7: 363-365.

Prasad, G., Singh, P.R. and Dayal, R., 1984. Studies in nematophagous fungi. VII. Two new records from India. *Proceedings of the National Academy of Sciences, India: SectionB: Biological sciences.*

Prasad, G., Singh, P.R. and Dayal, R., 1984. Studies in nematophagous fungi. VII. Two new records from India. *Proceedings of the National Academy of Sciences, India: Section B: Biological sciences*.

Reddy, P.P., 1983. *Plant nematology*. Agricole Publishing Academy, New Delhi, India, pp.287.

Rifai, M. A. and Cooke, R. C. 1966. Studies on some didymosporous genera of nematode trapping hyphomycetes. *Trans. Br. Mycol. Soc*., 49: 147-168.

Roubaud, H. E. and Deschiens, R. 1939. Capture des larves infectieuses de nematodes pathoogenes par des champignons predateurs due sol. *C.r. Acad. Sci., Paris,* 208: 245-247.

Rush MC, Lee F. 1983. Rice sheath blight: a major rice disease. *Plant Dis* 67:829–832

Ryker TC. 1939. The *Rhizoctonia* disease of Bermuda grass, sugarcane,rice, and other grass inLouisiana. *Congress International Society*; 6:198-201.

Sachchidananda, J. 1965. Studies on soil fungi III. Nematophagous fungi of agricultural soil. *Madras Agricultural Journal,* 51:524.

Sachchidananda, J., Rama Krishnan, K. 1971. Nematophagous fungi of agriculatural soil. *Mycopathology and Mycology Applied*, 43:235-241.

Sachchidananda, J., Swarup, G. 1966. Nematophagous fungi in Delhi soil. *Indian Phytopathology,* 19:279-285.

Sachchidananda, J., Swarup, G., 1967. Additional nematophagous fungi from Delhi soil. *Current Science,* 36: 677-678.

Sakhuja, P.K., Singh, I., Kang, L.S. and Kang, M.S., 1990. *Pl. Dis. Res*., **5**: 182-185.

Sharma, S.B., Sharma, H.K. and Pankaj. 2000. Nematodes problem in India In: Crop pest and Disease management- challenges for the millennium (Ed.D.Prasad and S.N. Puri), JoytiPublishers, New Delhi, pp. 267-275.

Siddiqi. Z. A. and Mahmood, I. 1997. Biological control of plant parasitic nematodes by fungi: *A review*. *Bio Resource Technology*, 58 (3): 229-239.

Simon, S., 2011. Management of root knot disease in rice caused by *Meloidogyne graminicola* through nematophagous fungi. *Journal of Agricultural Science*, *3*(1), p.122.

Sinclair JB. 1970. *Rhizoctonia solani*: special methods of study. In: Parmeter J (ed) *Rhizoctonia solani* biology and pathology. *University of California Press, Berkeley, Los Angeles and London*, pp 199–217.

Singh J, Mishra KK, Singh AK. 2013. Current status of web blight of mung bean. *An Asian journal of soil science.*;8(2):495-504.

Singh, K. P., Jaiswal, R. K., Kumar, N., Kumar, D. 2007. Nematophagous fungi associated with root galls of rice caused by *Meloidogyne graminicola* and its control by *Arthrobotrys dactyloides* and *Dactylaria brochopaga*. *Journal of Phytopathology*, 155, 193–197.

Singh, R.N. 1972. Root-knot disease of urd and mung in India. *Indian Journal ofMycology and Plant Pathology*, **2:** 87.

Singh, U. B., Sahu, A., Sahu, N., Singh, B. P., Singh, R. K., Renu, Singh, D. P., Jaiswal, R. K., Sarma, B. K., Singh, H. B., Manna, M. C., Subba, Rao, A., Prasad, Rajendra S. 2013. Can endophytic *Arthrobotrys oligospora* modulate accumulation of defence related biomolecules and induced systemic resistance in tomato (*Lycopersicon esculentum* Mill.) against root knot disease caused by *Meloidogyne incognita*. *Applied Soil Ecology*, 63, 45–56.

Singh, U. B., Sahu, A., Sahu, N., Singh, R. K., Renu, Ratna Prabha, Singh, D. P., Sarma, B. K., Manna, M. C. 2012a. Co-inoculation of *Dactylaria brochopaga* and *Monacrosporium eudermatum* affects disease dynamics and biochemical responses in tomato (*Lycopersicon esculentum* Mill.) to enhance bio protection against *Meloidogyneincognita*. *Crop Protection*, 35, 102–109.

Singh, U. B., Sahu, A., Sahu, N., Singh, R. K., Renu, Singh, D. P., Manna, M. C., Sarma, B. K., Singh, H. B., Singh, K. P. 2012b. *Arthrobotrys oligospora* mediated biological control of diseases of tomato (*Lycopersicon esculentum* Mill.) caused by *Meloidogyne incognita* and *Rhizoctonia solani*. *Journal of Applied Microbiology*114, 196–208.

Singh, U. B., Sahu, A., Singh, R. K., Singh, D. P., Meena, K. K., Srivastava, J. S., Renu Manna, M. C. 2012c. Evaluation of biocontrol potential of *Arthrobotrys* oligospora against *Meloidogyne graminicola* and *Rhizoctonia solani* in Rice (*Oryza sativa* L.). *Biological Control*, 60, 262–270.

Singh, U.B., Singh, S., Khan, W., Malviya, D., Sahu, P.K., Chaurasia, R., Sharma, S.K. and Saxena, A.K., 2019. *Drechslerella dactyloides* and *Dactylaria brochopaga*

mediated induction of defense related mediator molecules in tomato plants pre-challenged with *Meloidogyne incognita*. *Indian Phytopathology, 72*(2), pp.309-320.

Singh, U.B., Singh, S., Malviya, D., Chaurasia, R., Sahu, P.K., Sharma, S.K. and Saxena, A.K., 2020. *Drechslerella dactyloides* and *Dactylaria brochopaga* mediated structural defense in tomato plants pre-challenged with *Meloidogyne incognita. Biological Control, 143,* p.104202.

Slepetiene, J., Mackevic, N. and Tepliakova, T. 1993. A comparative characteristic of the effect of predatory fungi and nematicides on soil nematodes. *Acta-Parasitologica-Lituanica*, 24: 47-57.

Soprunov, F. F. 1958. Predacious hyphomycetes and their application in the control of plant pathogenic nematodes. *Acad. Sci. Turkmen SSR. Ashabed 1-365* (English Translation) 1966. *Israel Programm for Scientific Translation.*

Soprunov, F. F. and Galiulina, E. A. 1951. Predatory hyphomycetes from Turkmenistan soil. *Mikrobiologia*, 20: 489-499.

Srinivasachary Willocquet L, Savary S. 2011. Resistance to rice sheath blight (Rhizoctonia solani Kuhn.) [(teleomorph: thanatophorus cucumeris (A.B. Frank) Donk.] disease: current status and perspectives. Euphytica 178:1–22.

Srivastava SS. 1981. Studies on some predaceous fungi. Ph.D. Thesis, *Banaras Hindu University.*

Srivastava SS and Dayal R. 1982. Fungal predators of nematodes *Monacrosporium. Indian Phytopathology;* 35:650-653.

Srivastava SS and Dayal R. 1983. Fungal parasites of nematodes *Harposporium. Indian Phytopathology;* 36 (3): 392-393.

Srivastava SS and Dayal R. 1984. Ring and knob forming fungal predator of nematode. *Indian Phytopathology;* 37:691-694.

Starr, J. L, Heald C. M, Robinson A. F, Smith R.G. and Krausz J. P. 1993. *Meloidogyne incognita* and *Rotylenchulus reniformis* and associated soil textures from some cotton production areas of Texas *J Nematol* 25: 895-99.

Stirling, G. R. 1979. Techniques for detecting *Dactylella oviparasitica* and evaluating its significance in field soils. *Journal of Nematology,* 11: 99-100.

Stirling, G. R., Smith, L. J. 1998a. Field test of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control

of root knot nematodes. *Biological Control*, 11:231-239.

Stirling, G. R., Smith, L. J., Licastro, K. A., Eden, L. M. 1998b. Control of root knot nematodewith formulation of nematode trapping fungus *Arthrobotrys dactyloides*. *Biological Control*, 11:224-230.

Subramanian, C. V. 1963. *Dactylella, Monacrosporium* and *Dactylaria*. *Journal of Indian Botanical Science,* 42: 291-300.

Taheri P, Gnanamanickam S, Höfte M. 2007. Characterization, genetic structure, and pathogenicity of *Rhizoctonia* spp. associated with rice sheath diseases in India. *Phytopathology*; 97:373–383.

Tarjan AC. 1960. Induction of traps of nematophagous fungi using *Panagrellus redivivus. Nature*; 183 (4715), 779-780.

Taylor TN, Hass H, Kerp H, Krings M and Hanlin RT. 2005. Perthecial ascomycetes from the 400 million year old Rhymnie chert: an example of ancestral polymorphism. *Mycologia*; 97:269-285.

Thorn, R. G., Barron, G. L. 1984. Carnivorous mushrooms. *Science*, 224:76-78.

Tzean S.S. and Estey R.H. 1978. Nematode-trapping fungi as mycopathogens. *Phytopathology*; 68: 1266–1270.

Wang, F. H., Xu, Q., Wang, B. B., Wang, K. Y., Xue, Y. J., Cai, B., ... & Cao, X. 2017. Isolation, identification and characterisation of the nematophagous fungus *Arthrobotrys thaumasia* (*Monacrosporium thaumasium*) from China. *Biocontrol Science and Technology*, *27*(3), 378-392.

White, T. J., T. D. Bruns, S. B. Lee and J. W. Taylor. 1990.Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. *In: PCR Protocols: a guide to methods and applications.* Eds., M.A. Innis, D. H. Gelfand,J.J. Sninsky, and T.J. White. Academic Press, San Diego, California.

Whiteny HS and Parameter JR. 1964. The perfect stage of *Rhizoctonia hemalis. Mycologia*; 56:114-118.

Wibberg D, Jelonek L, Rupp O, Hennig M, Eikmeyer F, Goesmann A, Hartmann A, BorrissR, Grosch R, Pühler A, Schlüter A. 2013. Establishment and interpretation of the genome sequence of the phytopathogenic fungus *Rhizoctonia solani* AG1-IB isolate 7/3/14. *Journal of Biotechnology*; 167:142–155.

Woronin MS. 1870. *Sphaeria lemaneae, Sordaria coprophila*, *Arthrobotrys oligospora*. Beitr. Z. Morphol. U. Physiol. d. Pilze III, Abhandal. Senkenb. *Nat. Ges.*, 7 : 325.

Wu W, Huang J, Cui K, Nie L, Wang Q, Yang F, Shah F, Yao F, Peng S. 2012. Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. Field Crops Res. 128:101–108.

Yang G, Li C. 2012. General description of *Rhizoctonia* species complex. In: Cumagun CJ (ed) *Plant Pathology*; pp 41–52.

Zopf W. 1888. Zur. Kenntnis der Infections-Krankheiten niederer Tiere. *Nova Acta Leop. Academy of Nature. Halle*, Vol. 52:7.

Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, Zhu LH. 2000. Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). *Theoretical and Applied Genetics*; 101:569–573.

LIST OF APPENDIXES

Appendix-A

Chemicals used in current study

- Agarose
- Bromophenol blue
- 6X loading dye
- Chloroform
- Cetyl Tri methyl Ammonium bromide (CTAB)
- dNTP's (Deoxy Nucleoside Triphosphate)
- Ethylene Diamine Tetra Acetic Acid (EDTA)
- Ethidium bromide (10 mg/ml)
- Ethyl alcohol
- chilled isopropanol
- Isoamyl alcohol
- Mercaptoethanol GR
- NaOH pellets (Sodium Hydroxide)
- NaCl (Sodium Chloride)
- Phenol
- Poly vinyl pyrrolidine
- Proteinase K
- RNase H
- Taq polymerase
- 100bp ladder

Appendix B

PREPARATION OF BUFFERS AND STOCK SOLUTIONS

1. **CTAB BUFFER 200ML**

   6.0 g CTAB (Hexa decetyl trimethyl- ammonium bromide)

   20.0 ml 1 M Tris pH 8.0

   8.0 ml 0.5 M EDTA pH 8.0 (Ethylene diamine tetra acetic acid)

   56.0 ml 5 M NaCl

   60.0 ml H20

   Make up the volume upto 200ml with distilled water and autoclave the solution, then add 4g PVP 40 (Polyvinyl pyrrolidone- vinyl pyrrolidinehomopolymer, MW 40,000)

   Adjusted pH to 8.0 by adding HCl and put into 65^{o}C in water bath for 30 min before use

2. **1M TRIS BUFFER (PH 8.0)**

   Dissolved 121.19 g of Tris base in 700 ml of distilled water. Adjusted pH to 8.0 by adding HCl. Adjust the volume to 1000 ml with distilled water.

3. **0.5M EDTA (ETHYLENE DIAMINE TETRA ACETIC ACID)**

   Dissolved 186.12 grams of EDTA, free acid in about 700 ml of distilled water and adjusted the pH to 8.0 with NaOH. The volume was made to 1000 ml with distilled water. Autoclave the solution for sterilisation before use.

4. **ETHIDIUM BROMIDE**

   Stock 20mg / ml was prepared by dissolving 1gm of ethidium bromide in 50 ml ofwater.

5. **Chloroform: Isoamyl alcohol (24:1)**

   24 ml of Chloroform was blend with 1ml of Isoamyl alcohol (24:1) and stored atroom temperature.

6. **Phenol: Chloroform: Isoamyl alcohol (25:24:1)**

   Phenol: 25ml Chloroform: 24ml isoamyl alcohol: 1ml

   all the components were mixed and stored at room temperature.

7. **TAE BUFFER (TRIS / ACETATE / EDTA) 50X STOCK SOLUTION**

   242 g Tris base

   57.1 ml Glacial acetic acid 100 ml 0.5 M EDTA (pH 8.0)

   Adjusted the pH to 5-8.5 with acetic acid and made up to the volume to 1000mlwith distilled water. Sterilized by autoclaving before use.

8. **TE BUFFER (PH 8.0)**

   10 mM TrisHCl

   1 mM EDTA

   2 ml of 0.5 M TrisHCl with pH 8.0 was mixed with 0.2 ml of 0.5 EDTA and madeup to the volume to 100 ml with sterile distilled water.

9. **6X GEL LOADING BUFFER** 0.25% (W/V) Bromophenol blue40% (W/V) sucrose in water

   0.25g of Bromophenol blue was mixed with 40g of sucrose and made up thevolume to 100ml with distilled water.

10. **RNASE PREPARATION**

    RNASE BUFFER A.1M Tris (pH 7.5)

    B. 5M NaCl

    Took 0.5ml of 1M Tris (final concentration 10mM) and 75 µl of 5M NaCl (final concentration 15mM) and made up the volume to 50 ml. Took 25 mg of ribonuclease H into a tube and added RNase buffer to make a final volume of 5 ml (so final concentration 5mg / ml). Kept the tube in a boiling water bath for 10 min, cooled and made aliquots of 1 ml in 1.5 ml Eppendorf tubes and stored at -20^0C

Appendix C

List of Equipment Used

- Agarose gel electrophoresis
- Autoclave
- Centrifuge
- DNA Thermal Cycler
- DNA Thermal Cycler
- Electronic balance
- Freezer of -20ºC
- Gel Documentation System
- Glass hooks
- Incubator
- Incubator shaker 37^0C
- Magnetic stirrer
- Microcentrifuge tubes
- Microwave oven
- Nano drop
- pH meter
- Pipettes
- Power supply unit
- UV- transilluminator

VITAE

Mr. Abhay Pratap Singh

Masters in Plant Pathology

Department of Plant Pathology

Banda University of Agriculture

and Technology, Banda Uttar Pradesh, India (210001)

Permanent address: 1593, Ram Nagar Khaga Fatehpur
Pin – 212655, UP India

Correspondence address: Alha Hostel, Banda, Department of Plant Pathology (CoA) Banda University of Agriculture and Technology, Banda, 210001, UP

Hobbies: Playing cricket, reading motivational book.

Email: - apsyadav005@gmail.com

Phone Number: - 6392212488

Academics	Passing Year	Institute Name	Board/University	Percentage /OGPA
High School	2014	S.V.M.I.C Khaga Fatehpur	U.P. Board	**86.5%**
Intermediate	2017	Pt. Deen Dayal Upadhyay Sanatan Dharma Vidyalaya Azad Nagar Kanpur-2	CBSE	**70.2%**
Graduation B.Sc. (Ag.)	2021	Kulbhaskar Ashram P.G College, Prayagraj	Prof. Rajendra singh (Rajju Bhaiya) university, Prayagraj	**73.61%**
Masters in Plant Pathology	**2023**	**Banda university of agriculture & tech. Banda**	**Banda University of Agriculture & Tech. Banda**	**-**

Place: BUAT,

Banda

(Abhay Pratap Singh)

Date:

……