Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L).

THESIS

SUBMITTED TO THE

Banda University of Agriculture & Technology, Banda-210001, Uttar Pradesh, India

By

Radha Krishan

Id.No -1699

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (AGRICULTURE)

IN

PLANT PATHOLOGY

2022

Dedicated

To my mother

Late Smt. Rekha Devi

Radha Krishan....

BANDA UNIVERSITY OF AGRICULTURE & TECHNOLOGY, BANDA

Dr. Dharmendra Kumar
Professor & Chairman
ADVISORY COMMITTEE

Department of Plant Pathology
Banda University of Agriculture & Technology, Banda-210001(U.P.)

Dated-

Certificate-I

This is to certify that the thesis entitled ''**Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L)**'' submitted 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), is a genuine record of bonafide research work carried out by **Radha Krishan, Id. No. 1699** under my guidance and supervision. The results of the investigation in this thesis 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.

ENDORSED:

08.09.2022

Dr. Dharmendra Kumar
Professor, Plant Pathology
Chairman, Advisory committee

08.09.2022

Head
Department of Plant Pathology

Certificate-II

We, the undersigned members of the Advisory Committee of **Mr. Radha Krishan**, **Id. No.1699** a candidate for the degree of **Master of Science (Agriculture)** in **Plant Pathology** have gone through the manuscript of the thesis and agreed that the thesis entitled''**Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L**)'' may be submitted in the partial fulfilment for award of the degree.

Dr. Dharmendra Kumar
Professor, Plant Pathology
& Chairman Advisory Committee

Endorsed:

Dr. Vivek Singh
Assistant Professor, Plant Pathology
Co Advisor

Dr. Rakesh Pandey
Professor, Entomology
Minor Co Advisor

Head
Department of Plant Pathology

Dr. Deo Kumar
Assistant Professor, Soil Science & Agri. Chemistry
Co Advisor

Dr. Narendra Singh
Professor, Agronomy
Registrar's Nominee

Certificate – III

This is to certify that the thesis entitled ''**Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L)**'' submitted by **Mr. Radha Krishan, Id. No. 1699** in partial fulfilment of the requirement for award of the degree of **Master of Science (Agriculture)** in **Plant Pathology, Faculty of Agriculture**, Banda University of Agriculture & Technology (Banda) was examined and approved on **12/09/2022.**

Dr. Dharmendra Kumar
Professor, Plant Pathology
Chairman, Advisory Committee

Prof. B. K. Sarma
Department of Plant Pathology
BHU, Varanasi
External Examiner

Endorsed:

Head
Department of Plant Pathology

Advisory Committee

Dr. Vivek Singh
Assistant Professor, Plant Pathology

(Dr. Rakesh Pandey)
Professor, Entomology

Dean,
College of Agriculture

(Dr. Deo Kumar)
Assistant Professor, Soil Science & Agri. Chemistry

(Dr. Narendra Singh)
Professor, Agronomy

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Banda ***Radha Krishan***

Date:

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LIST OF ABBREVIATIONS

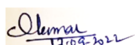
Symbol	**Abbreviations**
pH	Power of hydrogen
psi	Pounds per square inch
Spp.	Species
Microbes	Microorganisms
100x	100 times magnification
%	Per cent
Min	Minute
CMA	Corn meal agar
RPM	Revolution per minute
µl	Microliter
±	Plus, or minus
µm	Micrometer
^{0}	Degree
C	Centigrade
et al.	and others
Fig.	Figure
g	Grams
i.e.	That is
Hrs.	Hours
mm	Millimeter
ml	Milliliter
no.	Number
viz.	Namely
l	Liter
Sq.	Square

ABSTRACT

Name: **Radha Krishan** Id.No.: 1699
Semester: IVth Degree: M.Sc. (Ag) Plant Pathology
Year of admission: 2020 Department: Plant Pathology
Major: Plant Pathology Minor: Entomology

Thesis Title: "Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L)"

Drechslerella brochopaga and *Arthrobotrys musiformis* were isolated from the soil of the Banda district and characterized by using the relevant literatures. *Drechslerella brochopaga* and *Arthrobotrys musiformis* were found to capture and kill the nematodes by using constricting ring and adhesive network respectively. These fungi were tested for the ideal source of the substrate/ media for the growth and sporulation for. The best growth and sporulation of *Drechslerella brochopaga* was found on mung bean agar media and corn meal agar respectively. The best growth and sporulation of *Arthrobotrys musiformis* was noted on Lentil grain Agar medium followed by mix bran agar medium. The poor growth and sporulation of *Arthrobotrys musiformis* was noted on mung bean grain agar medium. Minimum growth and sporulation of *Drechslerella brochopaga* was found on mix bran agar and Lentil grain Agar medium respectively. Interaction of *Arthrobotrys musiformis* and *Drechslerella brochopaga* with *M. incognita* resulted in the formation of traps that subsequently capture and kill the *M. incognita*. Maximum trap formation (25.53) and trapping of *M. incognita* (J_2) were observed in *Drechslerella brochopaga* (98.83) followed by *Arthrobotrys musiformis*. *Drechslerella brochopaga* and *Arthrobotrys musiformis* were tested for their adoptability in soil of Banda district. *Drechslerella brochopaga* showed inhibitory effect of soil fungi stasis on germination (6.91-36.49% (but formed frequent conidial trap in vicinity of soil (57.95-87.57%). *Arthrobotrys musiformis* was generated by germ tube (75.92- 97.39 %) and no conidial trap formation was observed. Trapping of soil nematodes was also observed by the conidial trap of *Drechslerella brochopaga*. Bio efficacy test of *Arthrobotrys musiformis* and *Drechslerella brochopaga* in root-knot infested soil resulted in the reduction of number of root-knot (68.85%) and *M. incognita* (J_2) by *Drechslerella brochopaga*. *Arthrobotrys musiformis* reduced only 38.99% reduction in the number of root-knot and 50.76% reduction in *M. incognita* (J_2). The result of research carried out under the thesis indicates that *Drechslerella brochopaga* and *Arthrobotrys musiformis* were potential nematode-trappers and well adoptive in soil of Banda. However, the *D. brochcopaga* is more effective biocontrol agent because of its conidial trap forming ability, nematode-trapping ability and excellent bio-efficacy against *M. incognita*.



Dr. Dharmendra Kumar
Advisor

Radha Krishan
Author

शोध सारांश

नाम – राधा कृष्ण | आई.डी. न0 – 1699
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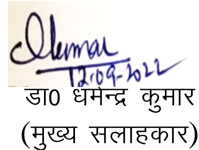
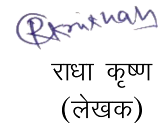
शोध प्रबंध का शीर्षकः *ड्रेस्लेरेल्ला ब्रोकोपेजा* और *आथ्रोबोट्रीस म्यूसिफॉर्मिस* की मिट्टी अनुकूलन क्षमता और बैंगन (*सोलनम मेलोंगेना* एल) पर *मेलोइडोगाइन इनकॉग्निटा* के खिलाफ जैव–नियंत्रण क्षमता पर अध्ययन

इस शोध प्रबंध के अर्न्तगत *ड्रेस्लेरेल्ला ब्रोकोपेजा* और *आथ्रोबोट्रीस* म्यूसिफॉर्मिस नामक सूत्रकृमि भक्षक कवकों को बाँदा जिले की मृदा से पृथक कर इनकी पहचान करने के उपरान्त इनकी मृदा में वृद्दि करने की योग्यता, विभिन्न पोषक माध्यमों पर इनकी वृद्दि योग्यता तथा बैगन कि मूलगांठ रोग के विरूद्ध इनकी जैव नियंत्रक क्षमता का अध्ययन किया गया है। *आथ्रोबोट्रीस म्यूसिफॉर्मिस* सूत्रकृमियों का भक्षण करने के लिए चिपचिपे फंद जाल का निर्माण करता है, जबकि *ड्रेस्लेरेल्ला ब्रोकोपेजा* त्रिकोशिकीय संकुचनशील वलय का निर्माण करके सूत्रकृमि भक्षण करता है। इन दोनो कवकों का विभिन्न कृत्रिम पोषक माध्यमों पर संवर्धन किया गया ताकि इनकी वृद्धि एवं कोनिडियम उत्पादन के लिए बेहतर संवर्धन भोज्य पदार्थ का पता लगाया जा सके। *ड्रेस्लेरेल्ला ब्रोकोपेजा* की सबसे अच्छी वृद्धि और बीजाणु उत्पादन क्रमशः मूंग बीन अगर मीडिया और कॉर्न मील अगर पर पाए गए। *आर्थ्रोबोट्रीस म्यूसिफॉर्मिस* की सबसे अच्छी वृद्धि और बीजाणु उत्पादन को मसूर दाल अनाज अगर माध्यम और उसके बाद मिक्स ब्रान अगर माध्यम पर पाया गया। मूंग बीन अनाज अगर माध्यम पर *आथ्रोबोट्रीस म्यूसिफॉर्मिस* की खराब वृद्धि और बीजाणु उत्पादन को नोट किया गया। *ड्रेस्लेरेल्ला ब्रोकोपेजा* की न्यूनतम वृद्धि और बीजाणु उत्पादन क्रमशः मिक्स ब्रान अगर और उसके बाद मसूर दाल अनाज अगर माध्यम पर पाए गए। प्रयोगशाला में दोनो सूत्रकृमि भक्षक कवको की जैव नियंत्रक क्षमता का पता लगाया गया जिसमें यह निष्कर्ष निकला कि *ड्रेस्लेरेल्ला ब्रोकोपेजा* सभी *मेलाइडोगाइन इनकोग्निटा* नामक सूत्रकृमियों की 98.33 प्रतिशत संख्या को मात्र 120 घण्टे के अन्दर भक्षण करता है जबकि *आथ्रोबोट्रीस म्यूसिफॉर्मिस* 120 घण्टों में मात्र 39.16 प्रतिशत सूत्रकृमियों का भक्षण करने में सक्षम होता है। मृदा में *ड्रेस्लेरेल्ला ब्रोकोपेजा* एवं *आर्थ्रोबोट्रीस म्यूसिफॉर्मिस* की क्रियाशीलता के अध्ययन से पता चला है कि *ड्रेस्लेरेल्ला ब्रोकोपेजा* के बीजाणु 57.95 से 87.57 प्रतिशत की संख्या में कोनिडियम सकुचनशील वलय या फन्द बनाते है तथा सूत्रकृमियो का भक्षण करते है। *आथ्रोबोट्रीस मुसीफोर्मिस* मृदा के बीजाणुओं ने 72.92–95.79 प्रतिशत की संख्या में मृदा में कवक तन्तु बनाकर अंकुरण किया परन्तु फन्द जाल का निर्माण नहीं किया। प्रयोगों से यह सिद्ध होता है कि यह दोनो कवक बाँदा कि मृदा में वृद्धि करने में सक्षम है। *आर्थ्रोबोट्रीस म्यूसिफोर्मिस* एवं *ड्रेस्लेरेल्ला ब्रोकोपेजा* कि जैव नियंत्रक क्षमता के अध्ययन से पता चला कि *ड्रेस्लेरेल्ला ब्रोकोपेजा* मृदा में 67.95 प्रतिशत मूल गांठ रोग के प्रकोप को कम किया जबकि *आथ्रोबोट्रीस म्यूसिफॉर्मिस* ने सिर्फ 39.99 प्रतिशत मूल गांठ रोग के संक्रमण को कम किया। इस शोध प्रबन्ध के अध्ययन से यह निष्कर्ष निकला है कि आथ्रोबोट्रीस *म्यूसिफॉर्मिस* और *ड्रेस्लेरेल्ला ब्रोकोपेजा* बाँदा जिले की मिट्टी में उपलब्ध है तथा यह अपनी जैव नियंत्रक क्षमता से बैगन के मूल गांठ रोग के संक्रमण को कम करने में सहायक हो सकते है। दोनों कवकों की जैव नियंत्रक क्षमता के मूल्यांकन से सि्द्व होता है कि *ड्रेस्लेरेल्ला ब्रोकोपेजा* द्वारा मिट्टी में बीजाणु फन्द जाल बनाने की क्षमता, सूत्रकृमियों का भक्षण करने और मूल गांठ रोग के विरूद्ध उच्च स्तरीय जैव नियंत्रक क्षमता के कारण *आर्थ्रोबोट्रीस म्यूसिफोर्मिस* की तुलना में अधिक प्रभावशाली जैव नियंत्रक है।





डा0 धर्मेन्द्र कुमार | राधा कृष्ण
(मुख्य सलाहकार) | (लेखक)

Chapter-I

INTRODUCTION

Brinjal (*Solanum melongena* L.), also referred as the eggplant, is a vegetable crop grown widely over the world, particularly in South Asia, and has Indian origins. It is a member of the Solanaceae family. with 92.7 g of moisture, 1.4 g of protein, 0.3 g of fat, 0.3 g of minerals, 1.3 g of fibre, 4.0 g of carbs, 124 international units of vitamin A, and 12 mg of vitamin C per 100 grams of edible portion, it has significant nutritional properties (Choudhary, 1983). The brinjal is considered to have "Ayurvedic" medical benefits, and white brinjal is particularly beneficial for diabetes people. Additionally, it has anthocyanins, which are antioxidant chemicals which defend the body from ageing, cancer, neurological disorders, and inflammation.

Plant parasitic nematodes are known to cause the global yield losses of 12.3% that results in an estimated $ 157 billion in losses per year (Abad *et al*., 2008). An annual loss to Indian agriculture of approximately 210 crores of rupees due to infection of plant-parasitic nematodes is estimated (Jain *et al*., 2007). Among the various species of plant parasitic nematodes, *Meloidogyne* spp. are reported to infect about 350 plant species in India, with *M. incognita* alone infecting over 250 genera of plants. To this crop's devastation, the losses brought on by the root-knot nematode *M. incognita* have been substantially greater than other plant-parasitic nematodes (Khushbu, 2016). Reddy and Singh (1981) reported that Root-knot nematode, *M. incognita* causes 33.68 % yield loss in brinjal.

Morphological as well physiological symptoms due to infection of root-knot nematode infections in plant roots are dramatic. Second-stage juveniles of root-knot nematodes develop from eggs and penetrate into the host's roots. As a result of nematode penetration and infection, large galls or knots can form throughout the root system of infected plants. Root-knot nematodes become immobile and finally develop into females in the host's roots (Starr *et al.,* 1993; Ogbuji, 2004 and Anwar *et al*., 2007). Severe infection results in reduced yield in brinjal. Studies have shown that root-knot nematode can cause suppression in yield of brinjal as high as 23 % (Sasser, 1979).

Plant-parasitic nematodes are difficult to manage than other pathogens because they often target the subsurface portions of plants and live in soil. The use of chemical nematicide against plant-parasitic nematodes is one of several methods for management of these

pathogens because it is efficient, simple to use, and shows results rapidly. However, it might also result in environmental pollution, a decline in soil fertility, and health risks for population living on this planet. As a result, biological management is currently being emphasised more since it is more practical, cost-effective, and ecologically safe.

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 parasitism, predation, competition, and antibiosis. Numerous organisms have been discovered to parasitize or prey upon nematodes, although bacterial and fungal antagonists were most frequently utilised in worm control and have drawn more attention (Krishanppa and Shreenivasa, 2009).

Nematode-trapping fungi are a group of soil-living fungi that forms adhesive or mechanical traps that capture, kill and digest the motile nematodes (Drechsler, 1937; Drechsler, 1941; Pramer, 1964; Thorn and Barron, 1984; Barron, 1977). Their traps are infectious structures that differs morphologically and characterized as adhesive knobs, adhesive nets, adhesive branches, non-constricting rings, and constricting rings. These fungi are found worldwide and attracted the attention of humankind due to its potential use as bio-control agents against plant-parasitic as well as animal parasitic nematodes (Kerry, 2000; Larson, 2000). In a variety of soil conditions, nematode-trapping fungi may be found living saprophytically and acting as facultative predators that capture the live nematodes (Pramer, 1964; Nordbring-Hertz *et al.*, 2006). These fungi forms traps on hyphae by which nematodes can be caught mechanically or by adhesion in order to transition to a nematophgaous lifestyle. As an alternative, conidial traps (CT) may develop immediately following conidial germination without a further hyphal phase (Persmark and Nordbring Hertz, 1997) or on short spore germlings (Cook, 1964; Kumar, 2003; Kumar *et al.*, 2015). These conidial traps are produced in response to soil nematodes, cow dung, and rhizosphere soil (Dackman and Nordbring-Hertz, 1992; Pressmark and Nordbring-Hertz, 1997; Kumar, 2003; Kumar *et al.*,2015). By forming conidial traps, nematode-trapping fungi may have the chance to capture, kill and extract the nematode's body's rich nourishment for growth and development despite fierce competition with other fungi for nutrients resources. By suppressing root-knot nematodes, promoting the accumulation of defence-related biomolecules, and inducing systemic resistance in plants, nematode-trapping fungi have been successfully used in soil to improve plant health (Stirling and Smith, 1998; Stirling *et al.*, 1998; Kumar and Singh,

2006a; Singh *et al*., 2007; Singh *et al*., 2012 and Singh *et al.,* 2013).

Drechslerella brochopaga Drechsler and *Arthrobotrys musiformis* are the two important species of nematode-trapping fungi that forms constricting rings and adhesive network respectively to capture, kill and digest the nematodes. *Arthrobotrys musiformis* was first observed by eminent mycologist Charls Drechsler in the year 1937 in isolation cultures Petri plates planted with pieces of decaying spinach roots collected near Norflok Va and has later been obtained in quantity also from samples of potting soil received from Florida as well as from serval lots of leaf moulds collected in deciduous woods in Vrginia. Aftger Dreshcler report, Linford (1937) also recorded *Arthrobotrys musiformis* among various nematode-capturing fungi found in Hawai. *Drechslerella brochopaga* was also first reported by Drechsler in the year 1937 as its species name *Dactylaria brochopaga*. Drechsler isolated *Drechslerella brochopaga* from the nematode infested culture plants following the addition of small quantity of leaf moulds collected from near Beltsville, Maryland, and also in leaf mods in deciduous woods in Arglington, Virginia.

Attempts to grow the nematode-trapping fungi on cheap substrates for its mass and delivery of these fungi in agricultural soil is most important for the biological control of plant-parasitic nematodes. There is a large possibility that the adoptability of nematode-trapping fungi may be affected by soil fungistasis, which inhibits the majority of fungal propagules (Dobbs and Hinson, 1953). Unfortunately, the majority of nematode-trapping fungi do not germinate in soil as a result of soil fungistasis (Cook, 1964; Mankau, 1962), which reduces their predicted capacity to kill nematodes. The most practical way to use these micro fungi on a wide scale seems to be the incorporation of nematode-trapping fungi as spore material into nematode infested soil (Mankau, 1962). To manage plant-parasitic nematodes, nematode-trapping fungi must be adoptive in agricultural soil.

Researchers have reported the importance of nematode-trapping fungi for the biological control of plant parasitic nematodes, however, the research on adoptability of these fungi in complex soil environment and screening of various substrates for its mass culture for biological control of plant parasitic nematodes have not been studied in detail. Therefore, keeping in view the of importance of nematode-trapping fungi in agriculture and seriousness of root-knot disease of brinjal, the present investigation was under taken with following objectives.

1. Isolation and characterization of *Drechslerella brochopaga* and *Arthrobotrys musiformis* and their *in vitro* potential test against *Meloidogyne incognita*.
2. Growth and sporulation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* on different media/substrates.
3. Growth and trap formation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* in different soil of Banda.
4. Performance test of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *M. incognita* on brinjal.

Chapter-II

REVIEW OF LITERATURE

2.1. History of root-knot nematodes

Berkeley (1855) first time noticed root-knots formation in cucumber roots in England. Cornu (1870) named it as *Anguillula marioni* but Müller (1884) named it as *Heterodera radicicola*. Goeldi (1887) observed root-knot nematode in coffee and coined the name *Meloidogyne* (Gr. = honey + female) but this was not accepted at that time. Until 1932, root-knot nematodes had been known as *Heterodera radicicola*.

Neal (1989) and Atkinson (1889) first time reported root-knot nematodes in North America. Barber (1901) first reported root-knot nematode in India on tea plants from Kerala. Chitwood (1949) re-established the genus *Meloidogyne* which was proposed by Goeldi. Chitwood described 5 species *(M. incognita, M. javanica, M. arenaria, M. hapla, M. exigua,* and re-described *M. exigua)* and 1 subspecies of nematodes (*M. incognita acrita*).

2.2. Yield Loss

Plant-parasitic nematodes are infamous to cause substatial economic losses to crop production (Luc *et al*., 2005; Sasser, 1990; Sikora and Fernandez, 2005; Taylor and Sasser, 1978). Sasser (1979) reported that *Meloidogyne spp*. attack more than 2000 species of plants. Root-knot nematodes are reported to cause annual losses in tropics up to 29% in tomato, 22% in okra, 24% in potato, 23% in eggplant, 25% in pepper, and 28% in beans.

Netscher and Sikora (1990) estimated the 14% annual global loss of most economically important crops such vegetables, fruits, and field crops amounting to over $80 billion annually. Nicol *et al*. (2011) reported the crop losses due to plant parasitic nematodes in the tropics and subtropics are 14.6% compared to 8.8 % in temperate regions. Koenning *et al*. (1999) reported the damage due to plant parasitic nematodes have exceeded $10 billion per year in the United States.

Tranier *et al.* (2014) studied the root-knot nematodes are microscopic roundworms, which cause severe agricultural losses. He also stated that biological control using microorganisms appears to be a better solution to reduce the quantity of pests infecting crops. Bhatti (1994) reported the yield loss of okra, tomato and brinjal suffered 90.9, 96.2 and 27.3% respectively due to *M. incognita* infection @ 3-4 larvae per gram of soil under field

conditions in India. The assessment of the Society of Nematologists Committee on crop losses indicates annual losses in the United States due to plan-parasitic nematodes to the tune of $ 1,038,374.300 in field crops, $225,145,900 in fruit and nut crops, $ 266, 989,100 in vegetable crops and $ 59,817,634 in ornamental crops (Anon, 1971). The losses associated with RKNs in small vegetable farms are not quantified but may range between 30-100% in tropical and subtropics. Yadav and Kumar (2016) estimated the occurrence of root-knot nematode disease on brinjal crops in five selected localities in and around Fatehabad, Agra. Highest frequency of disease occurrence in which almost all the roots have knot-like appearance (>85%) was reported from Firozabad Road area. Other localities were also having the significant infestations (62-80%) in brinjal crop.

2.3. Historical development of Nematode Trapping fungi

Arthrobotrys oligospora was first time reported by Fresenius's in 1852 as a common soil inhabitant. Woronin (1870) noted that *A. oligospora* produces hyphal nets, although he could not know the function of such network. Wilhelm Friedrich Zopf (1888) reported the hyphal nets of *Arthrobotrys oligospora* traps the migrating nematodes. Therefore, Zoph was the first scientist who discovered the biological connection between a nematode and a nematode-trapping fungus.

Charles Drechsler (1933, 1933a) demonstrated that the adhesive material produced by the hyphal nets of nematode-trapping fungi traps the nematodes. Drechsler's works on the nematode-trapping fungi between 1933 and 1975 motivated other researchers engaged in research on nematode-trapping fungi for biological control of plant and animal parasitic nematodes.

Duddington worked on nematode-trapping fungi during 1952–1972 and concluded that nematode-trapping fungi are often found in natural soils, agricultural soils, and all types of decomposing manures. He also published the methods for handling of nematode-trapping fungi (Duddington, 1955). His book "The Friendly Fungus" covers a various topics related to nematode-trapping fungi (Duddington, 1957). A number of researchers have made contributions to our understanding on nematode-trapping fungi (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).

Some preliminary works on nematode-trapping fungi were initiated in India 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 species of nematode-trapping fungi have been reported by several workers as 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*., 1984; Prasad, 1985). During research on nematode-trapping fungi, the taxonomy of the fungi has been a serious concern for scientists. Drechsler reported several nematode-trapping fungi species that capture, kill and parasitize 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-trapping fungi. A key of nematode-trapping fungi published by Cooke and Godfrey (1964) has been frequently used to identify the species of nematode-trapping fungi. The nematode destroying fungi, written by Barrron (1977), serves as a reference book for research on nematophagous fungi.

2.4. Research on trap formation and swelling of rings cells in nematode-trapping fungi

Couch (1937) reported that inoculation of nematodes to the pure culture of *D. bembicodes* resulted in the abundant formation of constricting rings. Commandon and de Fonbrune (1938) reported that inoculation of sterile nematode culture filtrates to a pure culture of the nematode-trapping fungus might cause the trap formation. Roubaud and Deschiens (1939) showed that human blood serum and extracts from earthworms were particularly more effective for induction of trap formation in nematode-trapping fungi. Pramer and Stoll (1959) coined the term 'Nemin' for the substance(s) inducing trap formation. They also proved that Nemin produced by nematode *Neoaplactona glaseri* act as triggering molecules for trap formation in nematode-trapping fungi.

Tarjan (1960) also reported formation of traps in nematode-trapping fungi by *Penagrellus redivivus* and stated that quantity of nematodes determines the quantity of trap formation. Feder *et al*. (1960) observed that a single dried nematode induced trap formation over a *Dactylella doedycoides*-containing 1 cm column which clearly indicated that an extremely small quantity of Nemin cause the trap formation.

Feder *et al*. (1963) suggested that the majority of Nemin present as endogenous Nemin in the nematode body. They studied the effect of endogenous nemin of *P. redivivus*

on morphogenesis of five species of *Dactylella* and noted differences in response of these nematode-trapping fungi to Nemin. *Dactylella cionopaga* exhibited the morphogenesis even at 10^{-6} dilution, whereas *D. bembicodes* and *Dactylella drechsleri* did not respond to 10^{-2} and 10^{-3} dilutions, respectively. In case of *Dactylella ellipsospora*, they noted that this fungus did not respond to endogenous Nemin but in contrast, it showed trap formation in culture in response to living nematodes. This indicates that endogenous Nemin and the extract of living nematodes were not identical in chemical composition.

Nordbring-Hertz (1973) reported that amino acid induce trap formation. She reported that several peptides were capable of inducing traps in *A. oligospora* and nutrition of a medium play critical role in trap formation. She further concluded that the peptides capable of inducing trap formation in nematode-trapping fungi were produced by biological degradation of proteinaceous material in the soil. Monoson *et al.* (1974) reported that endogenous Nemin extracted from five different species of nematodes induced trap formation.

Soprunov (1958) reported that spores of nematode-trapping fungi germinated in rainwater containing 1-2% ethyl alcohol results in the rapid formation of traps. He further reported that combination of CO_2 in rainwater with ammonium compounds formed ammonium carbonate, which induced trap formation. He also stated that sterilization of nematode filtrate caused the loss of morphogenic activity. Balan and Lechevalier (1972) reported that *A. dactyloides* also produce abundant traps in lack of nutrients or lack of water. Lawton (1957) reported the spontaneous trap formation on the hyphae of nematode-trapping fungi in culture. He noted that the spontaneous trap formation in *A. dactyloides* was stimulated by contact with glass and was not due to starvation of the fungus.

Bartnicki- Garcia *et al.* (1964) observed the CO_2 dependent trap formation in presence of Nemin in two strains of *A. conoides*. Though the optimum concentration of CO_2 was not determined, highest concentration (10%) produced maximum number of traps in one of the strains of the fungus. No traps were formed in absence of CO_2.

Commondan and de Fonbrune (1939) reported that the constricting ring of *D. brochopaga* could be inflated if the ring cells were rubbed with a glass micro needle. During inflation of individual ring cells, a number of small vacuoles increased in size and finally condensed to form a single large vacuole.

Muller (1958) observed that the stimulation of the inner wall of the constricting ring cell induced on instantaneous decrease in wall pressure and an increase in membrane permeability in that particular region of the wall. Due to the uptake of water from the surrounding sources, the level of osmotically active substances in the ring cell rose for the hydrolysis of the large molecule present, which maintain inflow of water. This process would continue until the swelling of ring cells was completed. In an alternative hypothesis, he suggested that osmotic recovery was rather slower. The cell would enable to expend by virtue of its initial osmotic potential, which fell during the expansion from level equivalent to 0.6 M sucrose to one finally equivalent to 0.2 M due to water uptake. The cell would then recover its initial osmotic potential after inflation by relatively slow hydrolysis of polymer present within it, and or by relatively slow transport of solute from the stock cells. He noted that the second version of the hypothesis was less physiologically exacting. It envisaged the stimulation of the cell as inducing a change in wall structure and a change in permeability, but did not require in addition any exceptionally rapid increase in the amount of osmotically active solutes in the cell.

Rudek (1975) reported that the air current caused the inflation of constricting ring cells. He pointed out that rings were in a dry condition and the only available water was by water imbibition through the stock and adjacent ring cell. He suggested that these imbibitions was overshadowed by the tremendous intake of water when the cells were submerged and he believed the complete closure could be explained in part by changes in membrane permeability.

2.5. Research on nematophagous behavior of nematode-trapping fungi

Cooke and Pramer (1968) stated that the presence of mycophagous nematode, *A. avenae*, did not affect the rate of colony growth of nematode-trapping fungi. They reported that the nematode population was not influenced by nematophagous activity of these fungi. At the initial phase, nematode population decreased due to the nematode-trapping activity of the fungi but later on predation became in low because of the toxic metabolites produced from nematodes, which eventually contributed to the death of these fungi. Due to this reason, nematode-trapping fungi were not visible after 30 days in dual culture with *A. avenae*.

Effect of temperature on growth and trapping effectiveness of some nematode-trapping fungi were studied by Monoson (1968). The major portion of his experiment dealt with the growth of *A. oligospora, A. musiformis, A. dactyloides, Monacrosporium*

bembicodes, M. cionopagum in relation to temperature and population of *A. avenae* and *Neotylenchus linfordi*. He noted the little fluctuation existed between increased trapping structures with temperature. Furthermore, too many nematodes did not encourage the growth of nematode-trapping fungi. His observation also supported the view of Cooke and Pramer (1968) that higher population of nematodes offered smaller amount of growth, which provided a negative correlation. Best predation of *A. oligospora, A. musiformis,* and *M. bembicodes* was observed at 15-20^{0}C against *A. avenae,* whereas *M. cionopagum* was most effective at 20^{0}C on V-8 agar medium and *A. dactyloides* at 15^{0}C on PDA, 25^{0}C on CMA and 30^{0}C on V-8 agar medium. However, all the nematode-trapping fungi trapped *N. linfordi* very effectively at all temperature regardless the media or the number of nematodes present in the inoculated sample. High percentage of nematode-trapping was noted in plates containing 100 nematodes.

Heintz (1978) reported that the age of fungus colony, number and type of nematodes added, and the ability of the fungus to form traps within the agar affected the trapping and parasitism of nematodes. He also calculated predacity index (PI) under different experimental conditions. Smaller was the PI value larger was the capacity of predation. Esser *et al*. (1991) studied interaction of *Dactylella megalospora* with 18 genera of phytonematodes and 9 free-living nematodes and reported that all the nematodes were trapped and assimilated by the fungus, however, some nematode exhibited resistance to trapping.

Belder and Jansen (1994) studied the *in vitro* assessment of nematophagous ability of *A. oligospora, M. cionopagum, A. dactyloides, A. scaphoides,* and *Duddingtonia flagrans* against *Meloidogyne hapla, M. incognita*, *Globodera pallida,* and *G. rostochinensis*. They observed that an isolate of *A. oligospora* and *M. cionopagum* were very much effective in capturing *M. hapla* and *M. incognita. Arthrobotrys conoides, A. dactyloides,* and *A. scaphoides* were intermediate in capturing efficiency. However, capture by some isolate of *Arthrobotrys* and *Duddingtonia* did not occur irrespective of the age of the mycelium. Further, they reported that the isolates, which were very much effective against *M. hapla and M. incognita,* had limited ability to capture the other nematodes e.g., *G. rostochinensis* and *G. pallida*. They concluded that nematode-trapping fungi have specificity in relation to the nematode-trapping ability.

Belder and Jansen (1994a) studied the effect of temperature, substrates, light, and aging of mycelia on the ability to capture *M. hapla* (J_2) by an isolate of *A. oligospora* (CBS - 29882) in *in-vitro* condition. They reported that capturing of nematodes by the mycelia of the fungus occurred within one hour irrespective of temperature and nematode mobility. However, development of traps was significantly lower at lower temperature (5-10^{0}C) than at higher temperature (15-30^{0}C). They also reported that nutritional conditions did not correlate to the efficacy in nematode hyphae attachment, but subsequent development of adhesive network was delayed on water agar. Aging of the fungal mycelia did not differ in capturing efficiency up to 70 days. Light, also did not have any influence on nematode capture.

de Gives *et al.* (1994) evaluated the nematophagous ability of *A. oligospora* and *A. conoides* against 3rd stage and 2nd stage larvae of *Haemonchus contortus* and *Nacobbus aberrans*, respectively *in vitro* at different temperatures. *Arthrobotrys oligospora* showed 35.87% and 25.71% trapping at 18^{0}C and 25^{0}C, respectively. None of the fungi was noted to produce three dimensional adhesive loops at 30^{0}C, hence, no capturing was observed. The nematode-trapping ability of *A. conoides* was higher than 90% for both nematodes.

Cayrol and Sawadogo (1991) observed that three celled constricting ring of the *M. bembicodes* trapped the nematode within one week of inoculation. They also noted the 80% inhibition in hatching in the presence of fungus.

Galper *et al.* (1995) developed the simple screening methods for assessing the nematophagous ability of nematode-trapping fungi and found that these fungi forms traps in response to *Caenorhabditis elegans*. They also noticed that the population of C. *elegans* was reduced by at least 90% within 3 days while the juveniles of *M. javanica* induced fewer traps and took more than three days to reduce more than 90% nematodes. They also observed that species such as *Dactylella candida* and *A. dactyloides* consistently reduced the number of *M. javanica* juveniles recovered from the soil, whereas network forming species *Monacrosporium* sp., *A. oligospora, A. conoides, A. musiformis* did not give the same results. They also introduced the agar "sandwich" and buried slide technique to monitor trapping activity in soil and concluded that *D. candida* and *A. dactyloides* consistently produced the traps within 5 days of being introduced, whereas network forming species produced fewer traps in the soil.

Cooke (1977) suggested that nematode-trapping fungi are able to capture and kill nematodes only for a relatively short period after the onset of decomposition of soil amendment. He observed that increasing the amendments above a certain level resulted in a decrease in parasitic activity despite an increase in nematode population. He concluded that nematodes were an alternative rather a sole nutrients source for nematode-trapping fungi. He also suggested that nematode-trapping fungi were active only during competition and the nematophagous habit was adopted to ease or escape competition for available substrate during decomposition of organic matter in soil.

2.6. Mass culture and formulations of nematode-trapping fungi

A nematode-trapping fungus *Arthrobotrys irregularis* is marketed in France as "Royal 350" (Caryol et al.,1978) for controlling root-knot nematodes in tomato (Cayrol and Frankowski, 1979). Commercial formulation of nematode-trapping fungus *Arthrobotrys robusta* var. *antipolis* is marketed in France as "Royal 300" (Cayrol,1983).

Grewal and Sahi (1988) developed a technique for rapid multiplication of *Arthrobotrys conoides* by soaking wheat grains in water for 20 minutes followed by 15 minutes boiling and drying. Chalks and gypsum were mixed with pieces of culture and incubated at 24°C.

Teplyakova *et al.* (1993) evolved a fungal preparation of Nematofungin BL using two strains of *A. oligospora* VKMF-3062 D, which was found effective against nematode problems in vegetable crops. Matskievich (1993) also observed that Nematofungin BL is effective against root-knot disease in cucumber.

Kumar *et al.* (2005) studied the variability in growth and sporulation of five isolates of a nematode-trapping fungus *Arthrobotrys dactyloides* on five culture media, 6 brans agar media, and 5 grains agar media. All the isolates recorded good sporulation on bran agar media except pigeon pea and lentil bran agar media. The grain agar media supported moderate to very good growth of all the isolates. In general isolate, B remained slow growing on these media except gram grain and sorghum grain agar media on which growth of this isolate was comparable to other isolates. Sporulation in general was good on all the grain agar media. Among different substrates screened, barley grain and pea bran were found superior to others for mass culture of *A. dactyloides*.

2.7. Fungistatic effect of soil on germination and conidial trap formation of Nematode-Trapping fungi.

Dobbs and Hinson (1953) reported that the spores of fungi in contact with soil showed inability to germinate under conditions, which might be favourable for spore germination.

Mankau (1962) studied the effect of soil fungistasis on *A. arthrobotryoides, A. dactyloides* and *Dactylella ellipsospora* by agar disk technique and observed that germinated spores of nematode-trapping fungi on soil were some times lysed or formed trapping organs on spores. He concluded that nematode-trapping fungi are poor competitors in the natural soil and their existence in soil environment depends on the successful capturing, killing and nutrient absorption from the nematodes body.

Cooke and Sathchuthananthavale (1968) observed the sensitivity of nematode-trapping fungi to soil fungistasis and found that with the exception of *A. musiformis*, all species showed sensitivity to fungistasis. Cooke (1964) categorized the nematode-trapping fungi into two broad groups an insensitive group (network forming) and sensitive groups (branch, knob, and ring formers). He reported that nematode trapping nature of these fungi was adopted to escape competition for substrates during decomposition of organic matter in the soil.

Cooke (1961) reported that addition of sucrose in soil stimulates nematode population and activity of nematode-trapping fungi. He also reported that decomposition of sucrose in soil reaches a certain stage at which fungi escape to trap the soil nematodes. Cooke (1962) reported that nematophagous activity of different nnematode-trappingfungi introduced into natural soil varies in both intensity and duration. He stated that the fungi forming constricting rings are very much effective in reducing nematode population in the soil than fungi forming adhesive trapping devices.

Persmark and Nordbring-Hertz (1997) examined the conidial trap forming ability of various fungi in the vicinity of soil and found that the conidial trap formation was greatest in *Arthrobotrys dactyloides* and *Monacrosporium gephyrophagum* followed by *A. superba* and *A. oligospora*. Kumar *et al.* (2015) also reported that conidia of *A. dactyloides* exposed to agricultural soils of India showed poor ger tube formation but formed frequent conidial traps, which captured and killed the nearby soil nematodes. Conidial traps, which trapped and kill the nematodes, grew well in all soils after nutrient absorption from nematode body.

2.8. Biological control of plant parasitic nematode by nematode-trapping fungi

Biological control of plant parasitic nematodes by nematode-trapping fungi was reviewed by many workers (Duddington, 1962; Mankau, 1980 and Siddiqi and Mahmood, 1997). Linford (1937) and Linford *et al.* (1938) noted a marked increase in the number of free-living nematodes in the soil and concluded that the increased population of nematode stimulated the population of nematode-trapping fungi that killed nematodes bringing them below the original level.

Linford and Yap (1938, 1939) reported that out of five species of nematode-trapping fungi introduced into the soil for biological control of plant parasitic nematodes, only few gave minor control of nematodes. However, when nematode-trapping fungi were introduced into soil with organic matter, they showed better bio-control. Ali (1990) evaluated the nematophagous activity of *A. oligospora* and organic amendments for the biological control of *M. incognita* on tomato and noted that when the *A. oligospora* was inoculated two weeks prior to transplanting and nematode inoculation, it gave 72% reduction in the number of root knots. Slepetiene *et al.* (1993) reported that *A. oligospora* was found more effective (46.5-81.9%) than the use of nematicides against root-knot caused by *Meloidogyne* sp. Colombo *et al.* (1995) tested a commercial formulation of the nematode-trapping fungi *A. oligospora* and *A. superba* containing mycelium and viable spores against root-knot disease of brinjal and reported that the formulation was effective in reduction of nematodes in soil, but could not reduce the number of root knots as compared to treatment with Fenamiphos. Arndt (1994) observed that *A. oligospora* and *A. dactyloides* reduced the population of *M. incognita* in tomato plant.

Stirling *et al.* (1998) reported that the formulations of *A. dactyloides* consistently reduced the numbers of *M. javanica* juveniles by more than 90%. They also observed that the field soils which were treated with granules (10g/litre) and planted to tomatoes, the number of galls induced by the root-knot nematode was reduced by 57-96%. Stirling and Smith (1998) reported that the formulations of *V. chlamydosporium* did not reduce galling while the formulations of *A. dactyloides* applied at 220-440 kg/ha substantially reduced the number of nematodes present in roots 4-8 weeks after planting.

Kumar and Singh (2006) reported that the introduction of mass culture of *A. dactyloides* in soil infested with 2000 juveniles of *M. incognita* per ‘kg’ before planting of tomato seedlings reduced the number of root knots by 5.6–45.6%, of females by 44.7–72.9%,

of egg masses by 44.5–51.3% and of juveniles by 37.9–81.8% and increased the plant growth in a pot experiment. The effect of this fungus as biocontrol agent was enhanced when its mass culture was applied with cow dung manure, which reduced the number of root knots by 61.7–66.6%, of females by 80.6–94.7%, of egg masses by 80.3–89.6% and of juveniles by 68.1–88.0%.

Singh *et al.* (2007) reported that the introduction of *A. dactyloides* and *D. brochopaga* in *Meloidogyne graminicola* infested soil respectively, reduced the number of root galls by 86% and of females by 94%, and eggs and juveniles by 94%. The biocontrol potential of these fungi also increased plant growth: shoot length by 42.7% and 39.8%, root length by 45.5% and 48.9%, fresh weight of shoot by 59.9% and 56.7%, and fresh weight of root by 20.3% and 25.1%, respectively, compared to these parameters for plants grown in *Meloidogyne graminicola* infested soil.

Kumar and Singh (2011) reported that the application of mass culture and spore suspension of the *D. brochopaga* with and without cow dung manure to soil infested with *M. graminicola* juveniles significantly reduced the number of root-knots, the number of egg masses, juveniles, and females compared to those in the control. Bio-efficacy of the fungus was heightened when the mass culture and a spore suspension were used in combination with cow dung manure,

Simon (2011) reported that application of mass culture of *Arthrobotrys oligospora* and *Dactylaria eudermata* reduced the number of root galls by 86.9% and 81.1%, of females by 94.2% and 91.7%. The mass culture of these fungi increased the plant growth: shoot length by 41.9% and 38.8%, root length by 44.6% and 41.8%, fresh weight of shoot by 61.1% and 58.7%, and fresh weight of root by 24.3% and 22.5%, respectively over nematode infested soil.

Singh *et al.* (2012a) reported that the application of *A. oligospora* in soil infested with *M. graminicola* and *R. solani* reduced the number of root-knot by 57.58–62.02%, sheath blight incidence by 55.68–59.32% and lesion length by 54.91–66.66% under greenhouse and miniplot (field) conditions.

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 74.57 mm cinnamic acid h^{-1} g^{-1} fresh wt.) after 10, 20 and 30 days of inoculation, respectively, in the greenhouse.

Singh *et al.* (2012c) reported that *Arthrobotrys oligospora* treated plants showed enhanced growth in terms of shoot and root length and biomass, chlorophyll and total phenolic content and high phenylalanine ammonia lyase activity in comparison with *M. incognita*- and *R. solani*-inoculated plants. They reported that *A. oligospora* has the potential to provide bio-protection agents against *M. incognita* and *R. solani*. They also suggested that the application of *A. oligospora* not only helps in the control of nematodes but also increases plant growth and enhances nutritional value of tomato fruits. Thus, it proves to be an excellent biocontrol as well as plant growth promoting agent.

Singh *et al.* (2013) studied the bio control potential of nematode-trapping fungus *Dactylaria brochopaga* against *Anguina tritici*, and *Meloidogyne graminicola* and reported that Co-inoculation of *D. brochopaga* and *C. anguillulae* significantly reduced the root-knot and seed gall in wheat and increased the plant growth parameters as compared to pathogen challenged plants without any bioagents/chemical nematicide. Singh *et.al (*2014) studied on *Catenaria anguillulae* and *Dactylaria brochopaga* for their capabilities to colonize wheat seed gall and also to reduce the *M. graminicola*, and *A. tritici* in wheat (*Triticum aestivum* L.). Co-inoculation of *D. brochopaga*, and *C. anguillulae* significantly reduced the root-knot and seed gall in wheat and increased the plant growth parameters including length, and dry weight of root and shoot as well as yield attributing characters like spike length; number of seed per spike, test weight etc. under greenhouse conditions as compared to pathogen challenged plants without any bioagents/chemical nematicide. Singh *et al.* (2019) investigated the role of *Drechslerella dactyloides* and *Dactylaria brochopaga* in reprogramming of root apoplast that enhance defence responses in tomato pre-challenged with *Meloidogyne incognita*. *D. dactyloides* and *D. brochopaga* were found most promising strains for control of *M. incognita*.

Singh *et.al* (2020) noted the multifarious effects of *D. dactyloides* and *D. brochopaga* when inoculated either individually or in combination in tomato plants pre-challenged with *M. incognita*. Additionally, *D. dactyloides* and *D. brochopaga* increased antioxidant as well as biocontrol activities significantly in tomato against *M. incognita*. Microscopic visualization of H_2O_2 and superoxide radicals in tomato leaves further corroborated the

above findings. Further, inoculation of *D. dactyloides* and *D. brochopaga* activated the phenylpropanoid pathway in roots leading to increase cell wall lignification's and pectin deposition in tomato roots in addition to direct trapping and parasitizing of juveniles and adults of *M. incognita*. From the results it can be concluded that increased cell wall lignification's and pectin deposition probably restricted the entry of nematodes and ultimately decreased the *M. incognita* population in tomato roots. It was also noted that plants treated with nematode-trapping fungi individually or in combination modulated the phenotypical alterations and assisted plant growth promotion.

MATERIALS AND METHODS

3.1. Isolation of *Drechslerella brochopaga*, *Arthrobotrys musiformis* and other nematode-trapping fungi

Isolation of *Drechslerella brochopaga, Arthrobotrys musiformis,* and other nematode-trapping fungi was done by the soil sprinkling technique originally described by Drechsler (1941) and developed by Duddington (1955) with slight modifications adopted by (Singh *et al.,* 2007, Kumar *et al.*, 2005) 500 g of soil samples were collected in separate polyethylene bags from the top profile of soil from different locations of Banda (Table-1). Sterilized corn meal agar medium (split corn grains: 20 g, agar-agar: 20 g, and distilled water: 1000 ml) cooled near the solidification was poured into several sterile Petri dishes to cover nearly 2/3rd area of a culture plate. After solidification of corn meal agar medium, melted and cooled rabbit dung agar (rabbit dung pellets -100 g, agar-20g, and distilled water-1000 ml) medium was poured into these Petri dishes to cover remaining area. Each soil samples were thoroughly mixed, sieved (2mm pore size) and nearly one gram of each soil sample was sprinkled over the poured medium into Petri dishes. For each soil sample, five Petri dishes were used as replicates. The Petri dishes were incubated at room temperature (25-30°C). Incubated Petri dishes were observed daily after one week of incubation for the occurrence of trap formation, capturing of nematodes, and formation of conidial heads of nematode-trapping fungi under binocular compound microscope. Spores of nematode-trapping fungi produced on single conidial heads near the trapped nematodes were picked under compound microscope with a sterilized fine needle and transferred into the corn meal agar medium separately for isolation of single species of nematode-trapping fungi. The spores inoculated Petri dishes were incubated at 25±1°C for growth and sporulation. After 10 days of incubation, spores of each fungal species were again transferred into Petri dishes containing corn meal agar medium. Further purification of different species of nematode-trapping fungi was made by single spore isolation. Pure cultures of each species of nematode-trapping fungi were maintained on corn meal agar medium at 25± 1°C.

3.2. Identification and characterization of *D. brochopaga* and *Arthrobotrys musiformis*

The size of conidia, conidiophore, hyphae, and trapping structures were measured by a calibrated ocular micrometer at different magnification (10×,20×, 40 ×, and 100 ×). For

calibration of ocular micrometer, a bright-field microscope, an ocular micrometer scale, a stage micrometer, and oil immersion were used. Ocular micrometer glass disc was inserted on the metal diaphragm and eyepiece was inserted in the microscope. Number of divisions of ocular and stage micrometers between the two coinciding lines was counted. 5 readings were taken with the high-power (10×,20×, and 40×) and oil-immersion objectives (100×). These observations are then used to calculate the calibration factor for the objective lens in use.

$$\text{One ocular division (in } \mu\text{m)} = \frac{\text{Number of divisions on ocular micrometer}}{\text{No. of divisions on stage micrometer}} \text{x} 10$$

After calibration, the ocular micrometer was used to measure the size of hyphae, conidiophores, conidia etc. in terms of length, breadth, and diameter by 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 shape and size of each morphological structures of the nematode-trapping fungi were recorded and compared with the original morphological descriptions of nematode-trapping fungi reported by Drechsler (1937), Drechsler (1950), Drechsler (1952), Drechsler (1954), and Cook and Godfrey (1964) for the purpose of identification of *D. brochopaga*, *Arthrobotrys musiformis* and other nematode-trapping fungi isolated during course of present investigation.

3.3 Pot culture of root-knot nematode *Meloidogyne incognita*

Pot culture of *Meloidogyne incognita* was maintained in the soil by regular planting of tomato and brinjal plants. Population of second stage juveniles of *M. incognita* was obtained from pot cultures of this nematode maintained on brinjal plants in the net house of the Department of Plant Pathology, Banda University of Agriculture and technology Banda. To obtain the population of second stage juveniles of *M. incognita*, several egg masses of *M. incognita* were picked from infected roots of brinjal plants and collected separately in cavity blocks containing sterilized distilled water. The cavity blocks containing egg masses were incubated at 25-30ºC for 3-4 days to get required population of *M. incognita.*

3.4. Assay of the nematophagous ability of *D. brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita*

In order to study the interaction between *D. brochopaga*, *Arthrobotrys musiformis,* and *Meloidogyne incognita* in dual culture, method described by Belder and Jansen (1994) was followed. 5 mm fungal discs of each species were taken from the periphery of 15 days old culture and inoculated into each of several Petri dishes containing corn meal agar medium (0.4 % corn and agar, 3mm thickness) in 50 mm Petri dishes. Inoculated Petri dishes were incubated at 27±1°C in dark condition. After 8 days of incubation, fungal discs were removed aseptically. A drop of sterile distilled water containing freshly hatched and thoroughly rinsed 200 *Meloidogyne incognita* (J_2) was inoculated with the help of a dropper into full grown cultures of *D. brochopaga* and *A. musiformis*. Petri dishes were incubated at 25±1°C and observations of formation of constricting rings and adhesive traps in 1.66 mm^2 fungal growth area in response to *M. incognita* (J_2) were noted daily up to 5 days under a research microscope. Data on number of traps in 1.66mm^2 areas were taken from center, middle and periphery of the fungal growth after nematode inoculation, and average number of traps formed on the surface or deep into the medium were noted. Observations on number of captured nematodes were recorded daily for 5 days of both fungal species and percentage of captured nematodes was calculated. Five Petri dishes were used as replicates. The interaction experiment was repeated twice, and pooled data were analyzed using two ways factorial C.R.D.

3.5 Growth and sporulation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* on different media/substrates

To study the growth and sporulation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* on some media/ substrates, mix bran (wheat, gram, pearl millet bran-20 gram), pure brans of wheat *(Triticum aestivum)* and grains of sorghum *(Sorghum bicolor*), wheat *(Triticum aestivum),* soybean *(Glycine max),* Chickpea *(Cicer arietinum),* Corn *(Zea mays),* Lentil *(Lens culinaris),* Moong Bean *(Vigna radiata),* and Barley *(Hordeum vulgare)* were taken. Each brans and grains were prepared @ 2% (each grain and bran- 20 g, agar- 20g, distilled water – 1000ml). Potato dextrose agar PDA; peeled potato – 200 g, dextrose – 20 g, agar – 20 g, distilled water – 1000ml) were also used as semi synthetic media. All media were prepared, sterilized and 20 ml of each medium was poured into several Petri dishes. 5 mm disc at fungal colony were taken from the periphery of 15 days old culture of

Drechslerella brochopaga and *Arthrobotrys musiformis* and inoculated into each Petri dishes. The inoculated Petri dishes were incubated at 27 ± 1ºC. Radial growth of *Drechslerella brochopaga* was measured at the interval of 3 days up to 18 days of inoculation. *Arthrobotrys musiformis* was measured at the interval of 3 days up to 9 days after inoculation. For observation on sporulation of each isolate in each medium, each Petri dish was flooded with 5 ml sterile water, agitated well for separation of spores, and collected separately. The spores were counted using heamocytometer and the number of spores per ml of water was noted for final calculation of average number of spores per plate. Five Petri dishes were used as replicates. The pooled data were analysed using two ways factorial C.R.D.

3.6. Assay of spore germination of *D. brochopaga* and *Arthrobotrys musiformis* in soil

For assay of germination, trapping devices formation and nematophgaous ability of *D. brochopaga* and *Arthrobotrys musiformis* in diverse condition of soil of Banda, method described by the Jackson (1958) was followed 500 g soil sample was collected from the top profile of soil from of various farms of Banda University of Agriculture and Technology and some villages of Banda district. Each soil sample was passed through a 2 mm sieve and thoroughly mixed before filling in Petri dishes. 50 g soil of each sample was placed in 90 mm Petri dishes and soil was watered near the full water holding capacity. Water agar blocks (10 mm size, 3 mm thickness) were placed directly on the soil surface. Two agar blocks were placed in a Petri dish at equal distance for inoculation of spores of two species of nematode-trapping fungi on each water agar block separately. The Petri dishes were incubated at room temperature (25-30 ^{0}C) for 24 hours to allow the diffusates to reach on the agar blocks. Conidial suspension of *D. brochopaga* and *Arthrobotrys musiformis* were prepared by harvesting the spores of each fungus by a fine needle. A large number of spores of *D. brochopaga* and *Arthrobotrys musiformis* were picked from a pure culture and put into a cavity block containing 0.5 ml of sterilized water. A small drop of spore suspension containing nearly 50-75 spores was placed on each water agar block and inoculated Petri dishes were incubated at room temperature (25-30^{0}C) for 24 hours for observation. Spore inoculated water agar block kept on clean slides in a moist chamber was treated as control. Agar blocks were removed from the soil by fine forceps and placed on the clean glass slides for observation. The base of the agar block was rinsed by distilled water to remove the soil

material from the base of agar block. The number of spores and germinated spores were counted. 12 replications were taken for each treatment and the percentage was calculated.

3.7. Mass Culture of *Drechslerella brochopaga* and *Arthrobotrys musiformis*

For the mass culture of *Drechslerella brochopaga* and *Arthrobotrys musiformis,* 35 grams of splitted sorghum were taken separately in 250 ml conical flask and moistened with 70 ml of water. The flasks were plugged with cotton and sterilized two times at 15 psi for 20 minutes. 10 mm fungal disc was cut from the periphery of the fungal growth of *Drechslerella brochopaga* and *Arthrobotrys musiformis* by a sterilized cork borer and inoculated in the centre of a substrate contained in a flask with the help of a sterilized inoculation needle. Five fungal discs were inoculated into each flask. The Inoculated flasks were incubated at 25ºC ad fully colonized grains with fungal growth of *Drechslerella brochopaga* and *Arthrobotrys musiformis* were analysed for counting of CFU g^{-1} of substrates. At the time of application in root-knot infested soil, the population of grain based formulation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* were 4.80×10^6 CFU g^{-1}.

3.8. Assessment of bio-control efficacy of *Drechslerella brochopaga* and *Arthrobotrys musiformis* fungi

The efficacy of sorghum grain-based formulations of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against root-knot disease was evaluated under net house at Banda University of Agriculture and Technology, Banda, India. Agricultural soil having 2000 J_2 of *M. incognita* per kg of soil was used for this experiment. Root-knot infected soil was thoroughly mixed with mass culture of *Drechslerella brochopaga* and *Arthrobotrys musiformis* @ of 4.80×10^6 colony forming unit/ kg of soil. The treatments were (1) Nematode-infested soil (control), (2) Soil inoculated with mass culture of *D. brochopaga* and (3) soil inoculated with mass culture of *A. musiformis*. Sorghum grain based formulations of *Drechslerella brochopaga* and *Arthrobotrys musiformis* were uniformly mixed in nematode-infested soil and filled in pots (1kg/pot). 35 days old brinjal seedlings free from root-knot infection were transplanted in one plant per pot. Treatments were replicated 10 times in a CRD. Data of the plant height, fresh shoot weight, number of root knots in each treatment, number of J_2 in each plant in each treatment were recorded after 36 days of planting. To count the number of *M. incognita* (J2), the roots of each plant were cut into small pieces and placed on modified Baerman dishes. The water level was maintained to touch the surface of tissue paper. The plates were incubated at room temperature (25-

30ºC) for 15 days and the water level was adjusted daily. After 15 days of incubation, the nematode numbers of each root system were counted. To count the numbers of *M. incognita* (J_2) in the soil, the method described by Southey (1970) for extraction of soil nematode was adopted. The total number of *M. incognita* (J_2) present in soil was added to the root population to represent the total number of nematodes per plant.

Statistical Analysis

All experiments were repeated twice. In the laboratory experiment, the data was analysed as a completely randomized design (CRD). Pot experiment was conducted in a completely randomized design (CRD) and data were subjected to analysis of variance (ANOVA) using the statistical OPSTAT programme. Data were compared $p \leq 5$.

Chapter-IV

EXPERIMENTAL FINDINGS

4.1. Isolation and identification of nematode-trapping fungi

During the routine isolation of nematode-trapping fungi from the horticultural and agricultural soil of Banda district of Uttar Pradesh, different species of nematode-trapping fungi were appeared in Petri dishes containing Corn Meal Agar and Rabbit Dung Agar media. The various species of 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 networks and constricting rings of nematode-trapping fungi. Morphological examination of conidia, conidiophores, hyphae, chlamydospores were done and size of each structures were measured. Each species of nematode-trapping fungi were identified on the basis of morphology of conidia, conidiophores, hypaes, 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 eudermata, Arthrobotrys cladodes,* and *Stylopege hadra* (Table-1).

Table-1 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 cladodes*	Horticulture farm BUAT, Banda
3	*Arthrobotrys eudermata*	Horticulture farm BUAT, Banda
4.	*Drechslerella brochopaga*	Aonla orchard Baberu and Mango orchard Banda.
5.	*Stylopege hadra*	Palahari village, Banda

Figure-1 (a-h): Characteristics of hyphae, conidiophore and conidia and constricting rings of *Drechslerella brochopaga*

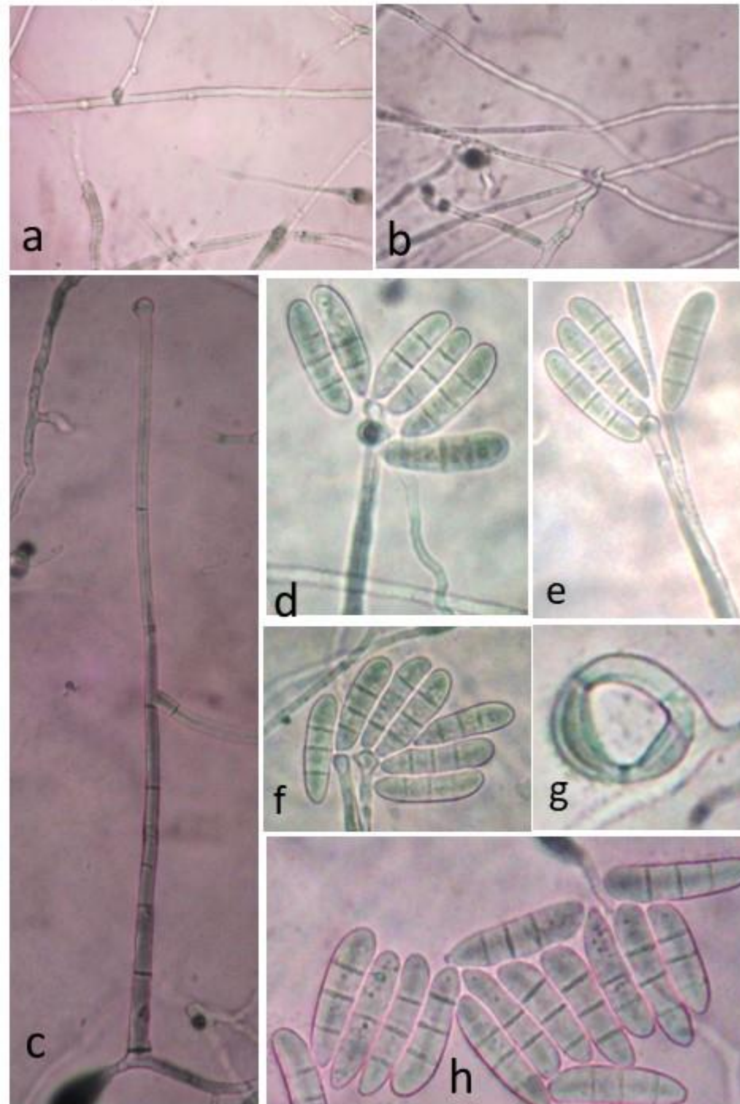


Figure-1- a &b: Hyphae of *D. brochopaga* (×450 & × 450) , c: Conidiophore of *D. brochopaga* (× 350), d & e: Conidia attached with conidiophore of *D. brochopaga* (×625), f: Conidial variant of *D. brochopaga* (× 515), g: Three celled constricting ring of *D. brochopaga* (×810) , h:Morphological variability in group of conidia of *D. brochopaga* (× 740)

Figure-2(a-i): Constricting rings of *Drechslerella brochopaga* and trapping and killing of nematodes

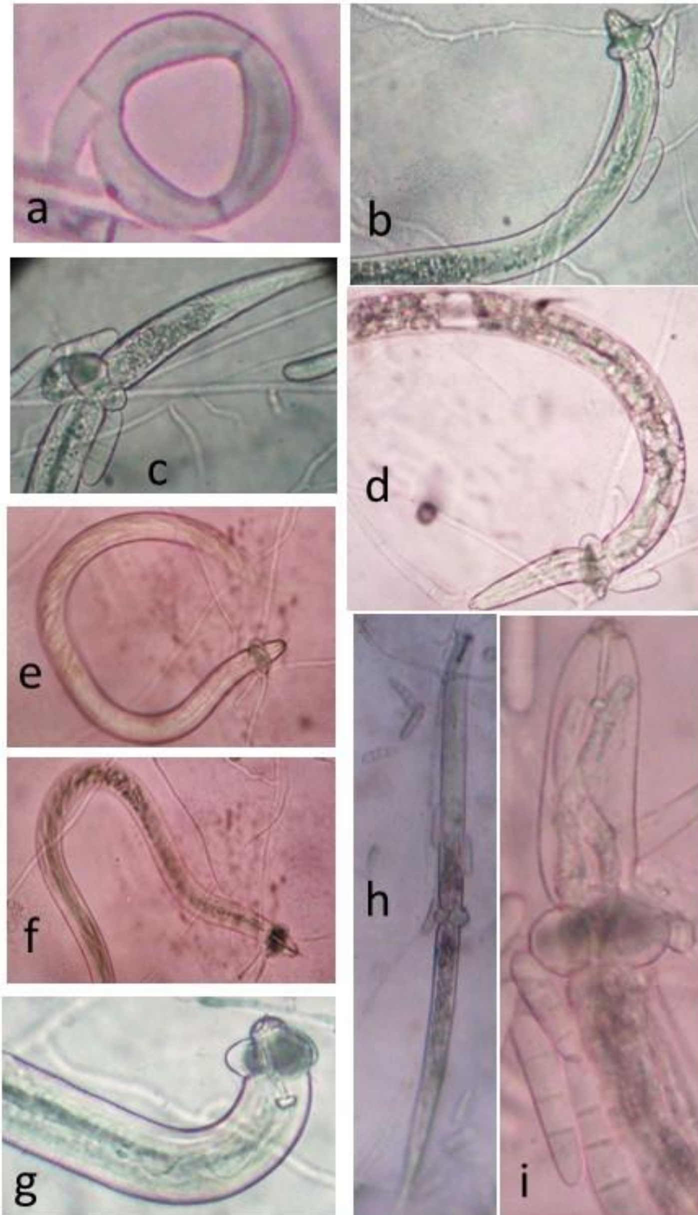


Figure-2- a: Enlarged view of three celled constricting rings of *D. brochopaga* (× 1100) , b-h: Trapping of free living and plant parasitic nematodes at head middle and tail region (× 430, × 440, × 330, × 330 , × 265, × 600, × 330) . i: Growth of hyphae of *D. brochopaga* in nematode body (× 800).

Out of the 5 species of nematode-trapping fungi isolated from the various soils of Banda, two species namely *Drechslerella brochopaga,* and *Arthrobotrys musiformis* were chosen for present research because of the different type of trapping organs and growth behaviour. *Drechslerella brochopaga* and *Arthrobotrys musiformis* were again purified with single spore isolation and maintained the corn meal agar media at regular subculturing at the interval of 15 days. Single spore cultures of *Drechslerella brochopaga, Arthrobotrys musiformis* were re-examined for their accurate identification of each species by measurement of conidia, conidiophore, hyphal characters, growth behaviour, etc. for final taxonomic resolution of these two species. The characteristics of *Drechslerella brochopaga* and *Arthrobotrys musiformis* are given below.

4.2. Characterization of *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- 4.6µm wide, conidiophores hyaline, septate, erect, 70-375µm, more typically 200- 350µm high, 4- 7µm wide at the base, tapering gradually upward to a width of 2.4- 3.5µm near the tip, there bearing on short blunt sterigmata 3- 12, mostly 3- 8 conidia in the beautiful radiating capitate 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 (Table-2). In presence of nematodes, the hyphae of the fungus developed constricting rings (Fig.1). Constricting rings produced on the conidia and on the hyphae 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 (Fig.-2) and grow inside the body region of nematodes. Constricting rings produced on conidia (conidial traps) were also found to capture and kill the nematodes (Fig.6).

Table-2. Morphological characteristics of *D. brochopaga* used in the present study

Characteristics features	**Name of the fungus -*D. brochopaga* Drechsler**
Mycelium	The mycelium of *D. brochopaga* on corn meal agar medium was spreading, hyphae hyaline, septate and 1.9-4.6 µm wide.
Conidial shape and features	Hyaline, straight or slightly curved, cylindrical or elongate, broadly rounded at the apex.
Conidial size	24-36µm ×5 - 9µm
Conidiophore length	Ranged from 70- 375µm but most typically 200 to 350 µm high in corn meal agar media.
Conidiophore width at base	4- 7µm
Conidiophore width at tip	2.4- 3.5µm
Trapping devices	Constricting ring
Chlamydospores	Absent

4.3. Characterization of *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 where 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 hyaline, ellipsoid, or slightly curved, broadly rounded at the wider distal end tapering toward the slightly protruded base 21-43µm, 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 globes or less frequently in mostly 15-21.5µm in diameter (Table-3, Fig.3&4).

Figure-3 (a-f): Characteristics of hyphae, conidiophore and conidia of *Arthrobotrys musiformis*

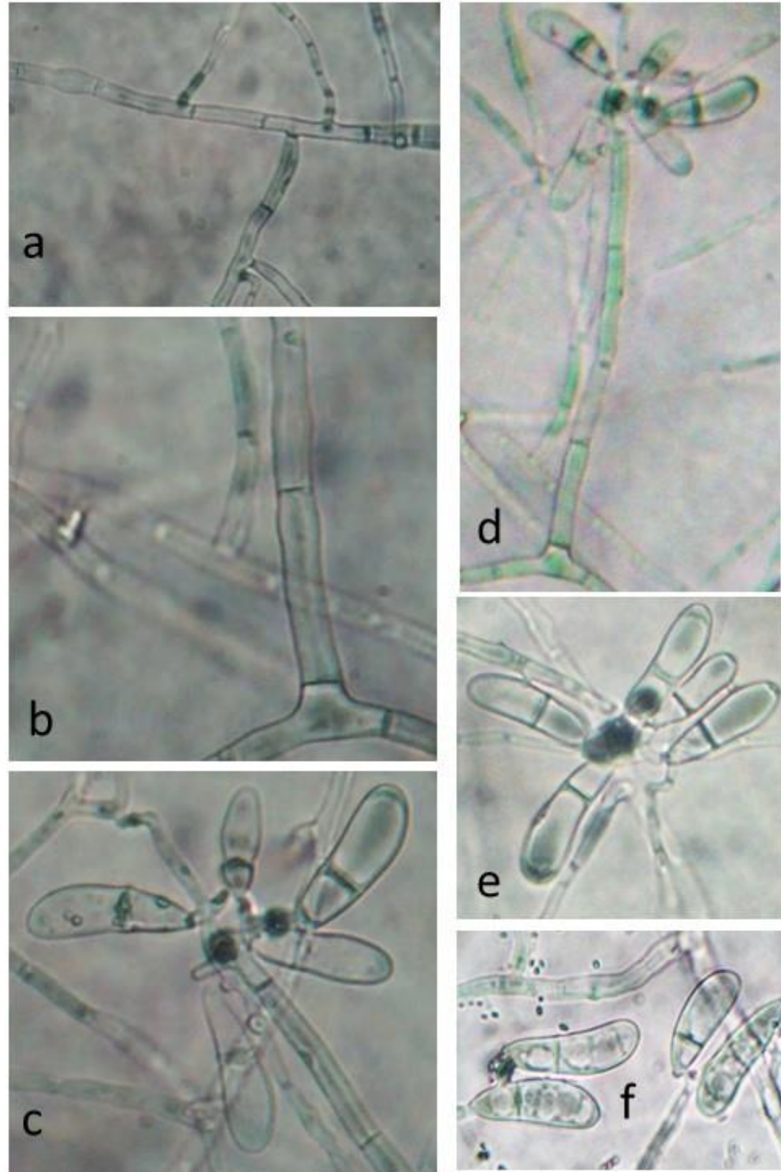


Figure-3- a: Hyphae of *A. musiformis* (× 400) , b-:Base of conidiophore of *A. musiformis* (× 1000), c: Enlarged view of conidia of *A. musiformis* attached with conidiophore (× 750), d: Conidia of *A. musiformis* attached on short conidiophore (× 520), e: Variable conidia attached with conidiophore (× 650), F: Morphological variability in conidia of *A. musiformis* (× 625)

Figure-4 (a-g): Characteristics and morphology of chlamydospores of *Arthrobotrys musiformis*

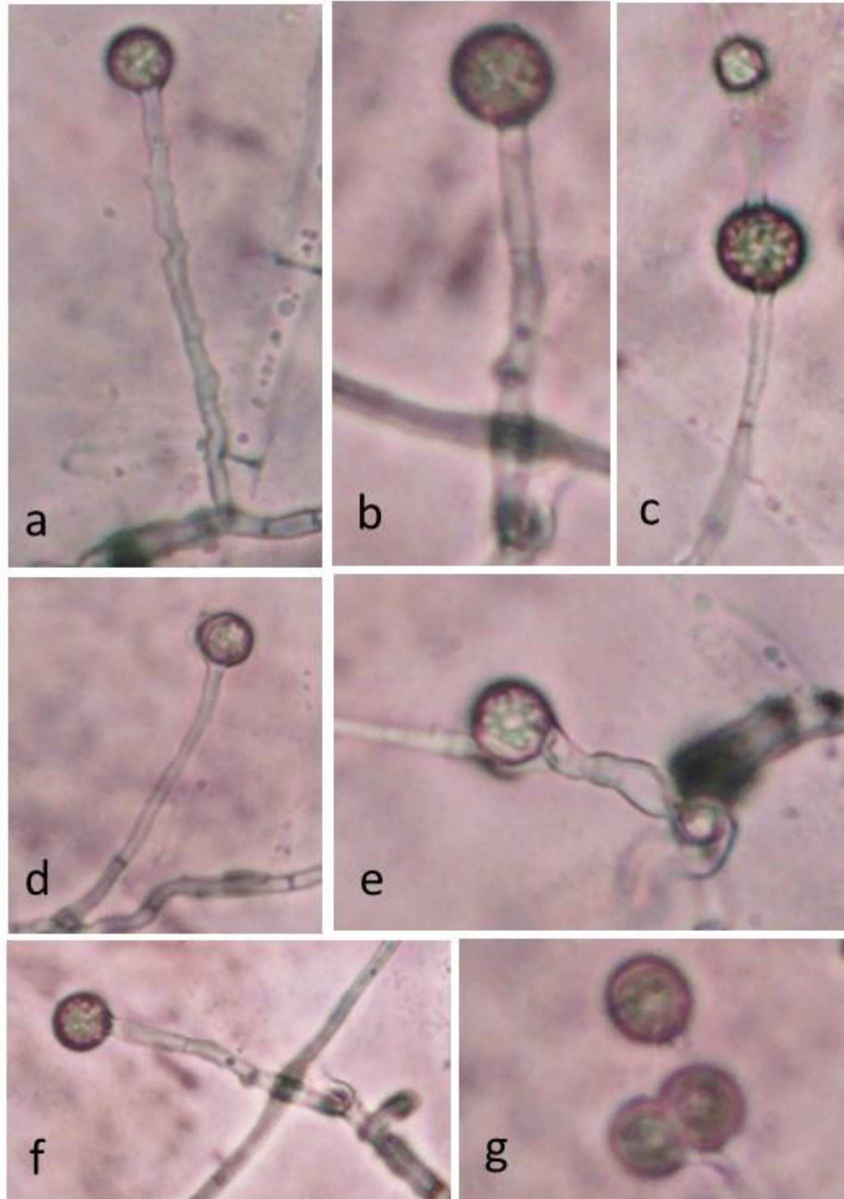


Figure-4- a –f: Morphological variability in chlamydospores of *A. musiformis* (× 550, × 800, × 700, × 450, × 700, × 450), g: Group of chlamydospores of *A. musiformis* (× 650)

Figure-5 (a-d): Adhesive trap formation and trapping of nematodes by *Arthrobotrys musiformis*

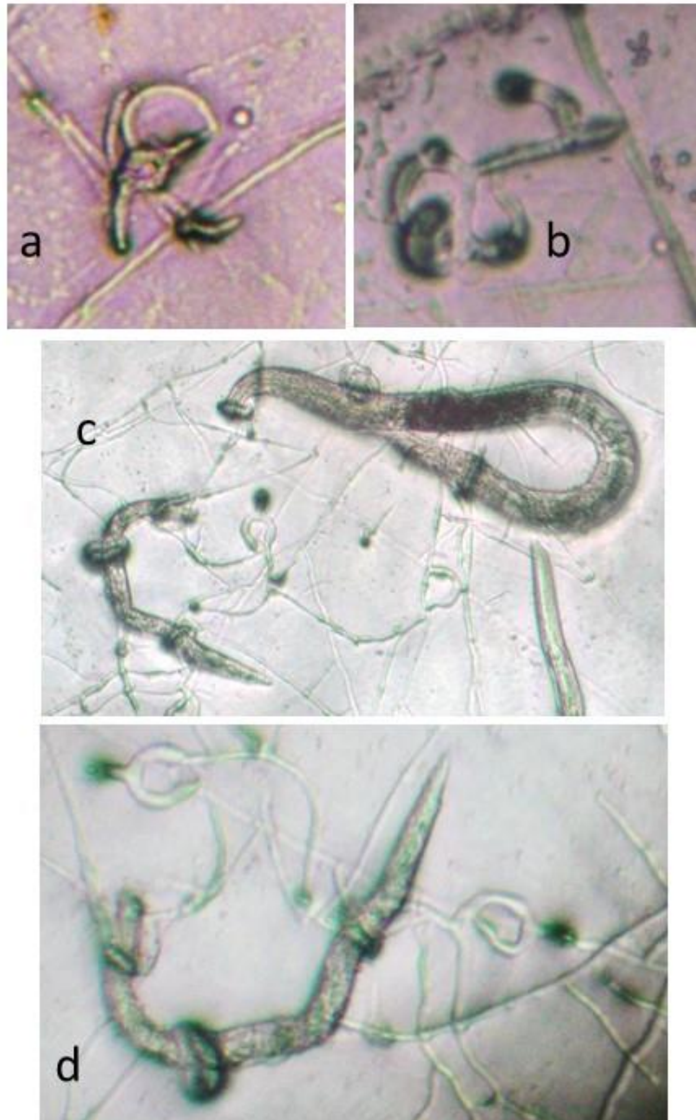


Figure-5- a & b: Adhesive traps of *A. musiformis* (× 330, × 330) , c &d: Trapping of nematode by adhesive traps of of *A. musiformis* (× 265 & × 330).

Table-3. Morphological characteristics of *A. musiformis* used in the present study

Characteristics features of fungi	**Name of the fungus- *A. musiformis* Drechsler**
Mycelium	Mycelium of *Arthrobotrys musiformis* were found spreading, vegetative hyphae hyaline, septate, mostly 2-8µm wide.
Conidial shape and features	Hyaline, ellipsoid straight or slightly curved, broadly rounded at the wider distal end, slightly protruded base.
Conidial size	21-43µm ×7.5-12.5µm
Conidiophore length	148-445µm
Conidiophore width at base	5-9µm
Conidiophore width at tip	2.5-4.0µm
Trapping devices	Adhesive traps
Chlamydospores	Present in old culture.15-21.5 µm in diameter.

Classification of *Drechsleralla brochopaga* Drechsler and *Arthrobotrys musiformis* Drechsler are given below:-

Kingdom	Fungi
Phylum	Ascomycota
Sub-Phylum	Pezizomycotina
Class	Orbiliomycetes
Order	Orbiliales
Family	Orbiliaceae

4.4. Growth and sporulation of *Drechslerella brochopaga* and *Arthrobotrys musiformis* on different media/substrates

Effect of different media/substrates on growth and sporulation of *Drechslerella brochopaga* is presented in Table-4. It is evident from the results that all the media and substrate supported slow colony growth of *D. brochopaga* in the media. *D. brochopaga* took 16 days to cover the full growth in 90 mm culture plates on best supported culture

media. All the media/substrates responses variable effect in terms of growth and sporulation of *D. brochopaga* on different culture media. It is evident from the result that the maximum growth of *D. brochopaga* was found in Mungbean grain agar followed by sorghum grain agar, chickpea grain agar, corn meal agar, lentil grain agar, soybean grain agar, wheat grain agar, PDA, wheat bran agar and barley grain agar. The lowest growth was observed on mix bran agar (bran of chickpea, wheat, and pearl millet) medium. Out of the 11 media/substrates tested for sporulation of *D. brochopaga*, maximum sporulation was observed in corn meal agar medium followed by soybean grain, mix bran, sorghum grain, barley grain, wheat grain, wheat bran, moong grain, chickpea grain agar, and PDA. The minimum sporulation was found on lentil grain agar medium. It was interesting to note that lentil grain agar media supported the good growth of the *Drechslerella brochopaga* but produced the lowest conidial production of the fungus (Line Graph-1&2).

The growth and sporulation of *Arthrobotrys musiformis* on different media and substrates are presented in Table-5. It is evident from the results that *Arthrobotrys musiformis* grew well in all the media with fast growth. However, the maximum colony growth was observed on lentil grain agar medium and mix bran followed by wheat bran, soybean grain agar medium, barley grain agar medium, corn meal agar medium, sorghum grain agar medium, chickpea grain agar medium, PDA, wheat grain agar medium and mung bean grain agar medium. Maximum sporulation of *A. musiformis* was found on lentil grain agar medium followed by mix bran agar medium, chickpea grain agar medium, sorghum grain agar medium, wheat bran agar medium, corn meal agar medium, barley grain agar medium, soybean grain agar medium, wheat grain agar medium, mung bean grain agar medium, and PDA. It was interesting to observe that and fluffy growth of mycelium was observed on PDA medium but the both fungi yielded lowest to minimum conidia production on this media. Low growth and sporulation or fast growth and sporulation of *A. musiformis* and *D. brochopaga* on different media may be attributed to the nutritive requirement of both the tested fungi and nutrient supplement present in different grains and brans agar media. It was interesting to note that some media which supported the good growth and sporulation of *A. musiformis* was not found good for growth and sporulation of *D. brochopaga* (Table-4-5; Line figure-1 -5). Kumar *et al.* (2005) reported the growth and sporulation of five isolates of *A. dactyloides* on different synthetic media and substrates and found that barley grain and pea bran brans media are superior to other media for growth and sporulation of this fungus.

4.5. Assessment of trap formation and carnivorous efficacy of *D. brochopaga* and *A. musiformis* against *M. incognita*

Inoculation of 200 freshly hatched second stage juveniles (J_2) of *M. incognita* into pure culture of *D. brochopaga* and *A. musiformis* in corn meal agar medium (0.4%) resulted in induction of constricting rings on the hyphae of *D. brochopaga* within 24 h. Adhesive nets in *A. musiformis* were not observed within 24 hours but few number of adhesive nets were observed on the hyphae of *A. musiformis* within 48 hours. Number of constricting rings and adhesive traps were increased with the passage of time. Increase in the average number of trapping structures in both fungi in response to *M. incognita* in 1.66mm^2 area were higher in *D. brochopaga* followed by *A. musiformis* (Table 6 & 7, Line graph-6&7). Higher number of trap formation was observed in *D. brochopaga* on 5th day of nematode inoculation per 1.66 mm^2 area of fungal growth. Second stage juveniles of *M. incognita* were freely moved in the media and captured by constricting rings of *D. brochopaga* and adhesive nets of *A. musiformis*. The trapped nematodes were digested completely after 48 hours of trapping by both the nematode-trapping fungi. *D. brochopaga* trapped and killed 98.33 % J_2 of *M. incognita* whereas *A. musiformis* captured only 39.16 % *M. incognita* (J_2) within 120 hours after nematode inoculation (Table-6&7, Line graph-6&7). *D. brochopaga* and *A. musiformis* captured and killed *M. incognita* at head, tail, and middle body region by constricting rings and adhesive nets respectively *A. musiformis* trapped the *M. incognita* by adhesion whereas *D. brochopaga* tightly trapped the nematodes by swelling of constricting ring cells. Results revelled that trap formation and trapping of nematodes had a strong relation for the nematode-trapping ability of *D. brochopaga* and *A. musiformis*. The ability of production of higher number of constricting rings in *D. brochopaga* in comparison to the adhesive loops of *A. musiformis* may be attributed to the response of these fungi to the morphogenic substances produced by the nematodes. Other possibility may be the genetic makeup of these fungi which make them able to be more efficient nematode trapper by formation of abundant trap formation and trapping of nematodes. Singh *et al.* (2007) also reported that constricting ring forming fungi are more able nematode trapper than adhesive trap forming species.

4.6. Adaptability of *D. brochopaga* in different soil of Banda

Conidia of *D. brochopaga* exposed to different soils of Banda district had poor germination without trap on germ tube (0.42-17.88%) with no further growth and germination inhibition ranged from 6.91-36.49% However, 57.95-87.57% conidia of *D.*

brochopaga placed over soil frequently germinated by conidial traps either directly on conidium or on short germ tube (0.0-1.79%) with no hyphal extension (Table-8, Fig. 6). No hyphae were formed from conidia-bearing traps in the close vicinity of soil. Further, no lysis of conidial traps was observed during 36 h. On water agar discs placed over various soils of BUAT campus Banda and villages soil, free living nematodes were usually attracted towards the conidial traps and were captured and paralyzed (Fig.6). The number of trapped and paralyzed nematodes on each water agar discs placed over each soil sample varied (0.0 to 7.0 on each agar blocks). The maximum nematode capturing and paralysis was recorded on agar discs placed over soil collected from soil beneath neem tree. It was interesting to observed that after trapping and killing of the nematodes, the cells of a spore produced hyphae on which traps were formed which were fully functional to capture the nematodes. It was also noted that conidial traps, which did not capture the nematodes because the low population of nematodes failed to grow and proliferate in soil due to fungistatic effect of soil. The results indicate that *D. brochopaga* is a poor saprobe but survive in nematophagous phase by trapping and killing of free-living nematodes in fungistatic soil environment provided, the soil harbours good number of nematodes. The formation of traps on growing hyphae after trapping, paralysis and extraction of nutrients from parasitized nematodes indicates that the *D. brochopaga* can grow and multiply in nematophagous phase until the nematodes are available in soil as a food source for this fungus.

4.7. Adaptability of *A. musiformis* in different soil of Banda

The adaptability of *A. musiformis* in different soil of Banda were tested to know the growth and development of this fungus under the complex environment of soil. It is evident from the result that conidia of *A. musiformis* placed in close vicinity of soil germinated with variable frequency which ranged from 75.92-97.39% (Table-9). However, further growth and conidial trap formation were not observed during 36 h. of observations. similarly, no trapping of nematodes was observed on agar disc placed on soil. The result indicates that the conidial germination of *Arthrobotrys musiformis* was inhibited with a range from 2.60-24.07 by fungistatic effect of soil. No conidial trap formation and trapping of nematodes were recorded during the 36 hours of spore inoculation. The result indicates that *A. musiformis* is adoptive in soil due to minor to moderate inhibition in spore germination in complex soil condition of Banda.

4.8. Assessment of bio-control efficacy of *Drechslerella brochopaga* and *Arthrobotrys musiformis* fungi

Application of *D. brochopaga* and *A. musiformis* in the soil infested with 2000 juveniles of *M. incognita* caused significant reduction in number of root-knots and number of J_2 after 5 weeks of planting. Maximum per cent reduction in number of root-knots and second stage juveniles were 67.85% and 78.50% respectively by application of *D. brochopaga*. Application of *A. musiformis* in the root-knot infected soil caused 38.99 % and 50.76 % reduction in number of root knots and juveniles of *M. incognita* respectively. Both fungi increased the shoot length of brinjal plant in comparison to plant growth in sick soil alone (Table-9). It is evident from the observation that both the nematode-trapping fungi trapped and killed the infective second stage juveniles (J_2) in the root-knot infested soil at resulted in the reduction of root-knot disease in brinjal plant.

Figure-6 (a-i): Germination of *A. musiformis* and conidial trap formation and trapping of soil nematodes by *Drechslerella brochopaga* in vicinity to soil

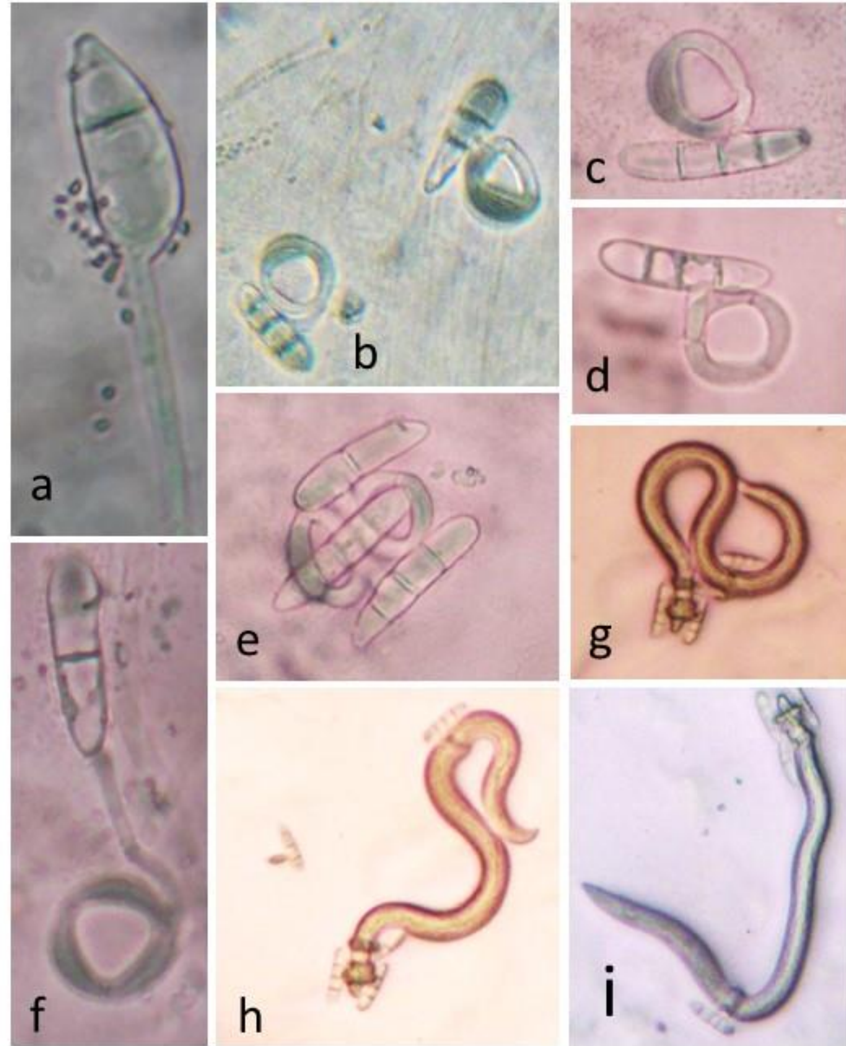
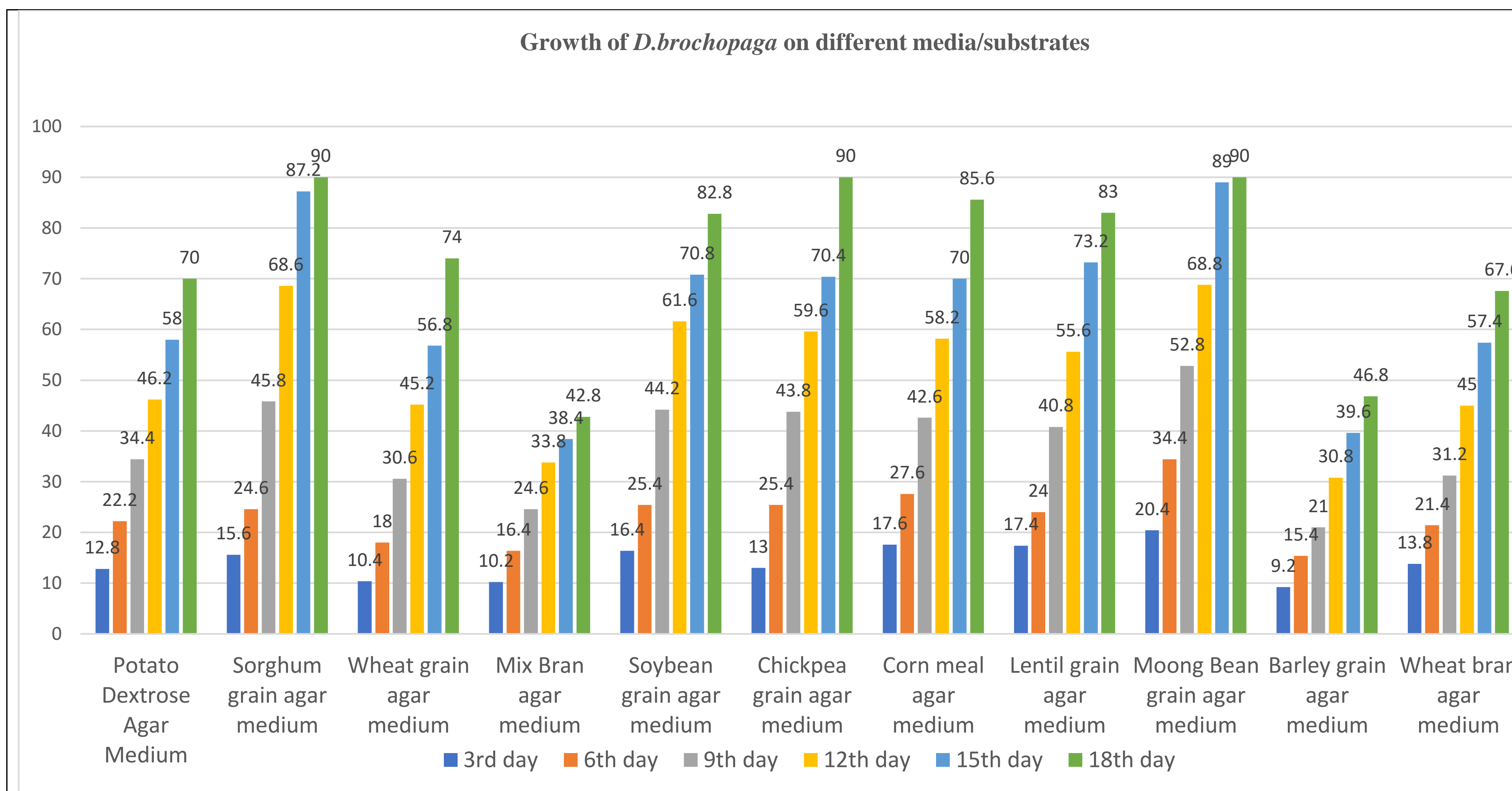


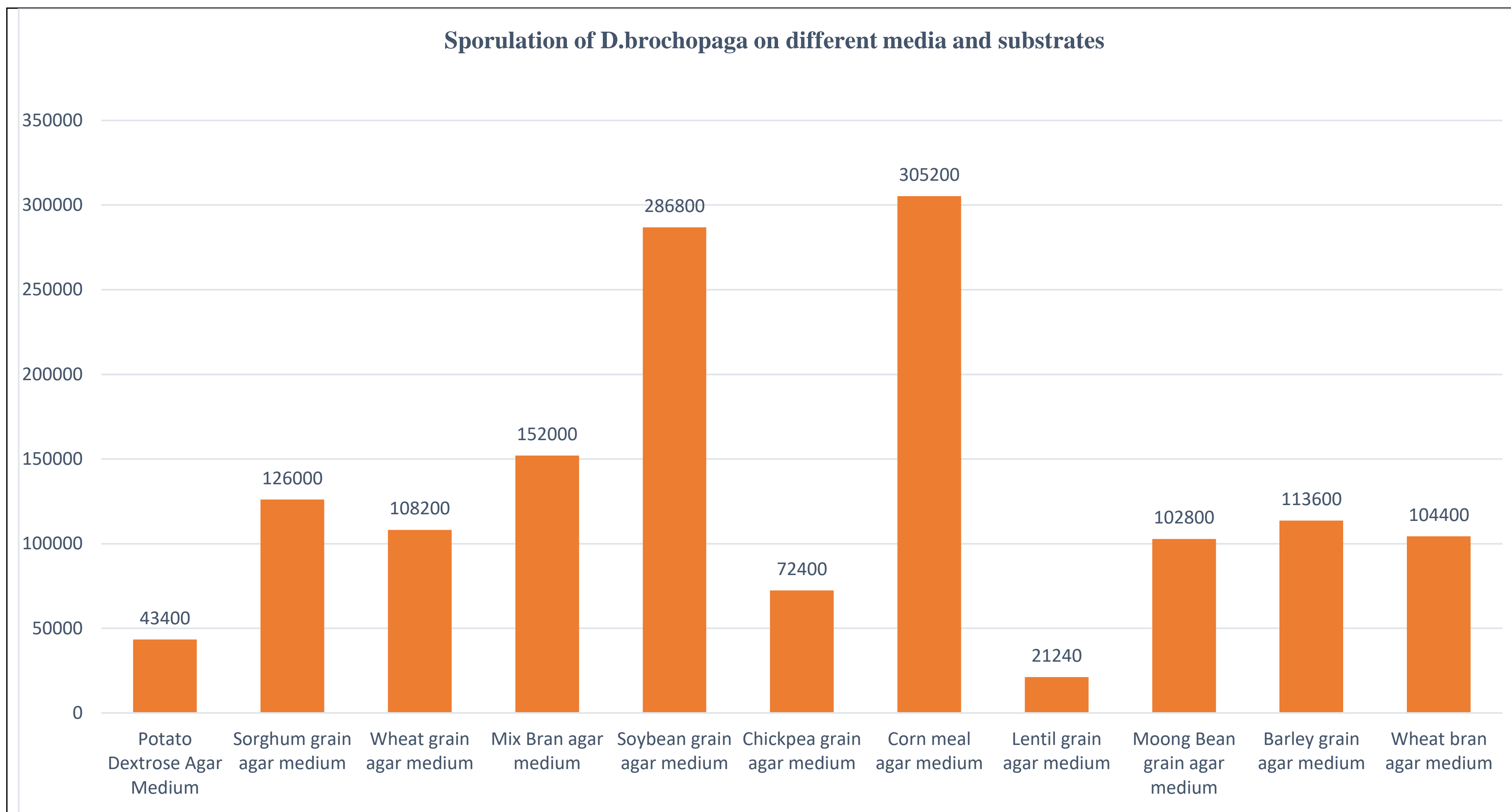
Figure-6- a: Spore germination of *A. musiformis* in vicinity to soil (× 975) , b: Conidial traps formation in vicinity to soil (× 655) c-e: Enlarged view of conidial trap in vicinity to soil (× 914, × 225, × 225) f: Trap formation in conidial germ tube, h & i: Trapping of nematode by conidial traps of *D. brochopaga* in vicinity to soil (× 225, × 225).

Table: 4 Effect of different media on Growth and Sporulation of *Drechslerella brochopaga* on different media/ substrates

Media	**Radial growth (mm) *Drechslerella brochopaga* on different growth period**							**Sporulation /Culture plates**
	3rd day	**6th day**	**9th day**	**12th day**	**15th day**	**18th day**	**CD @ 5%**	
Potato Dextrose Agar Medium	12.8	22.2	34.4	46.2	58.0	70.0	**2.33**	43400
Sorghum grain agar medium	15.6	24.6	45.8	68.6	87.2	90.0	**2.29**	126000
Wheat grain agar medium	10.4	18.0	30.6	45.2	56.8	74.0	**1.56**	108200
Mix Bran agar medium	10.2	16.4	24.6	33.8	38.4	42.8	**3.39**	152000
Soybean grain agar medium	16.4	25.4	44.2	61.6	70.8	82.8	**1.46**	286800
Chickpea grain agar medium	13.0	25.4	43.8	59.6	70.4	90.0	**3.28**	72400
Corn meal agar medium	17.6	27.6	42.6	58.2	70.0	85.6	**2.50**	305200
Lentil grain agar medium	17.4	24.0	40.8	55.6	73.2	83.0	**3.49**	21240
Moong Bean grain agar medium	20.4	34.4	52.8	68.8	89.0	90.0	**2.15**	102800
Barley grain agar medium	9.2	15.4	21.0	30.8	39.6	46.8	**2.58**	113600
Wheat bran agar medium	13.8	21.4	31.2	45.0	57.4	67.6	**2.11**	104400
CD @ 5%	**1.618**	**2.15**	**2.66**	**2.76**	**2.54**	**2.87**		**31,640.560**



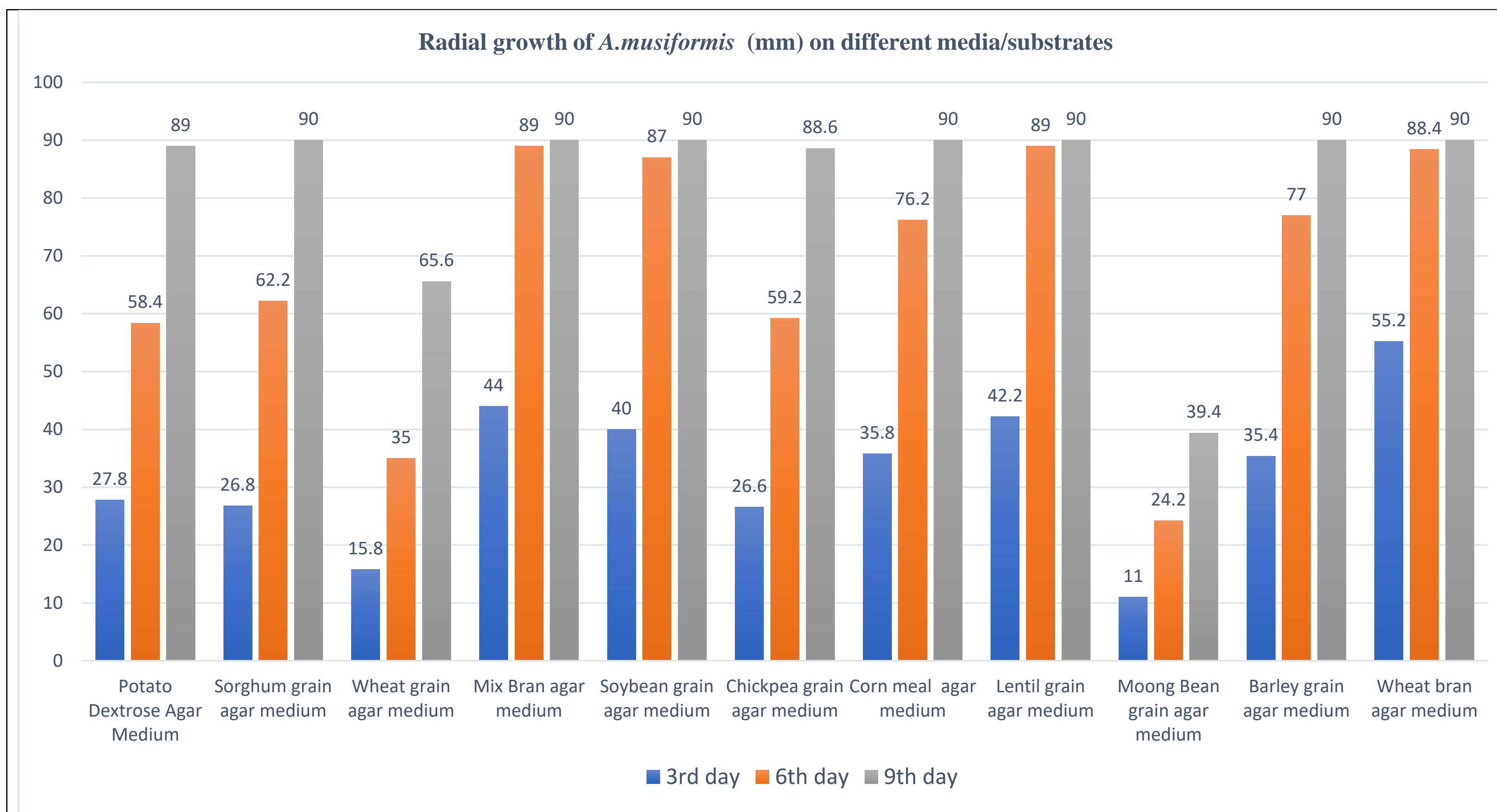
Line Graph -1. **Effect of different media/substrates on growth (mm) of *Drechslerella brochopaga* during different growth period.**



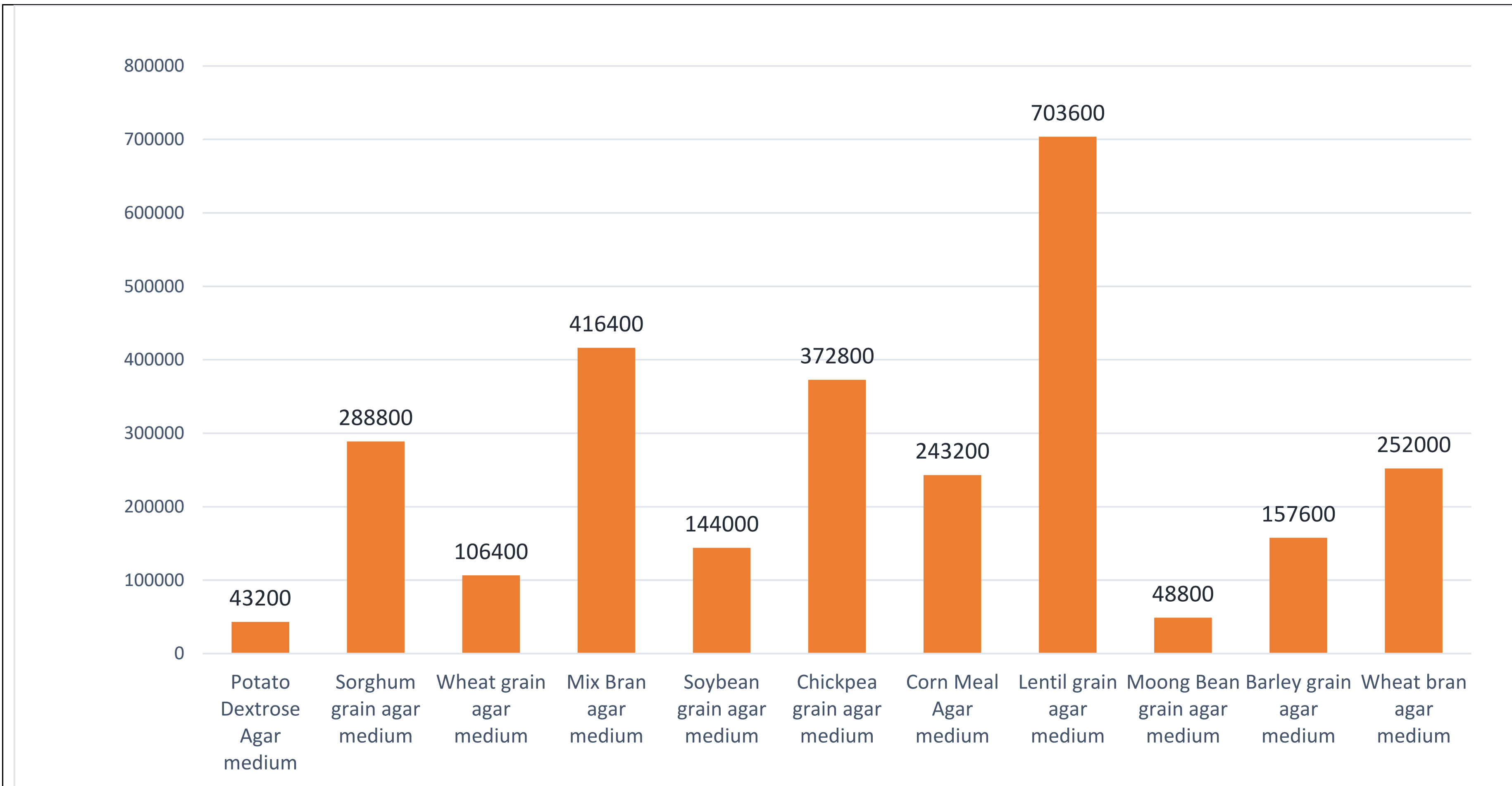
Line Graph -2. Effect of different media/substrates on sporulation of *Drechslerella brochopaga.*

Table. 5 Effect of different media on Growth and Sporulation of *Arthrobotrys musiformis*

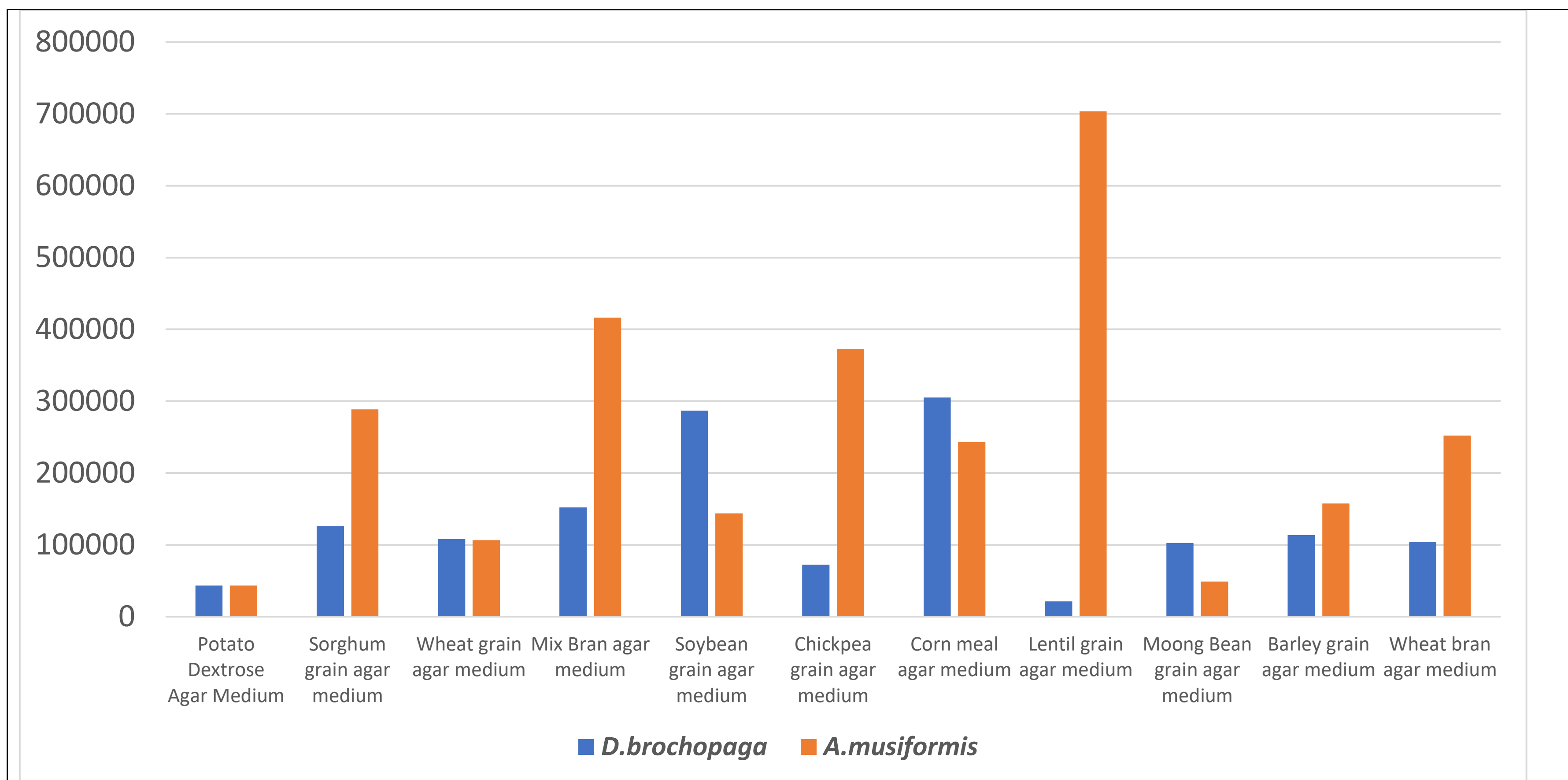
Culture Media/Substrates	Growth of *Arthrobotrys musiformis* (mm) during different date of observations			CD @ 5%	Sporulation (Conidia production /culture plate)
	3rd day	6th day	9th day		
Potato dextrose agar medium	27.8	58.4	89.0	**2.413**	43200
Sorghum grain agar medium	26.8	62.2	90.0	**4.684**	288800
Wheat grain agar medium	15.8	35.0	65.6	**2.607**	106400
Mix Bran agar medium	44.0	89.0	90.0	**2.728**	416400
Soybean grain agar medium	40.0	87.0	90.0	**3.267**	144000
Chickpea grain agar medium	26.6	59.2	88.6	**3.346**	372800
Corn meal agar medium	35.8	76.2	90.0	**1.869**	243200
Lentil grain agar medium	42.2	89.0	90.0	**4.117**	703600
Moong Bean grain agar medium	11.0	24.2	39.4	**1.505**	48800
Barley grain agar medium	35.4	77.0	90.0	**2.988**	157600
Wheat bran agar medium	55.2	88.4	90.0	**3.010**	252000
CD @ 5%	**3.22**	**3.511**	**1.150**		**40,253.54**



Line Graph: 3- Effect of different media /substrates on growth (mm) of *Arthrobotrys musiformis* during different growth period



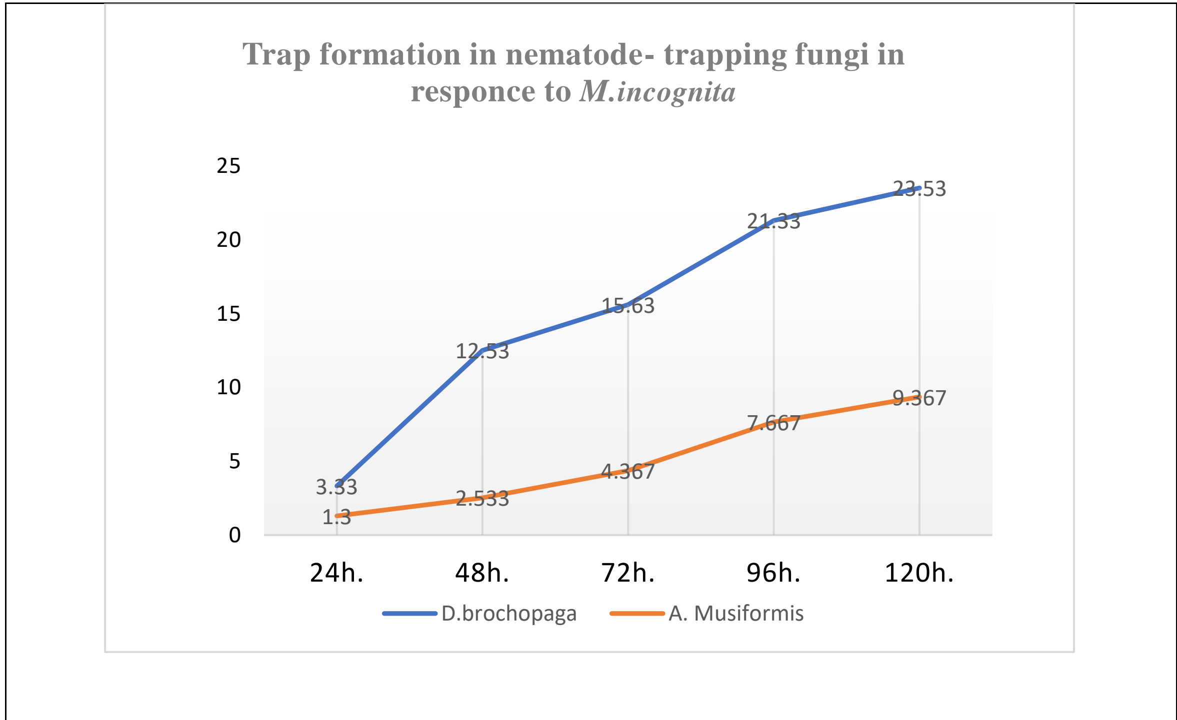
Line Graph -4. Effect of different media/substrates on Sporulation of *Arthrobotrys musiformis*.



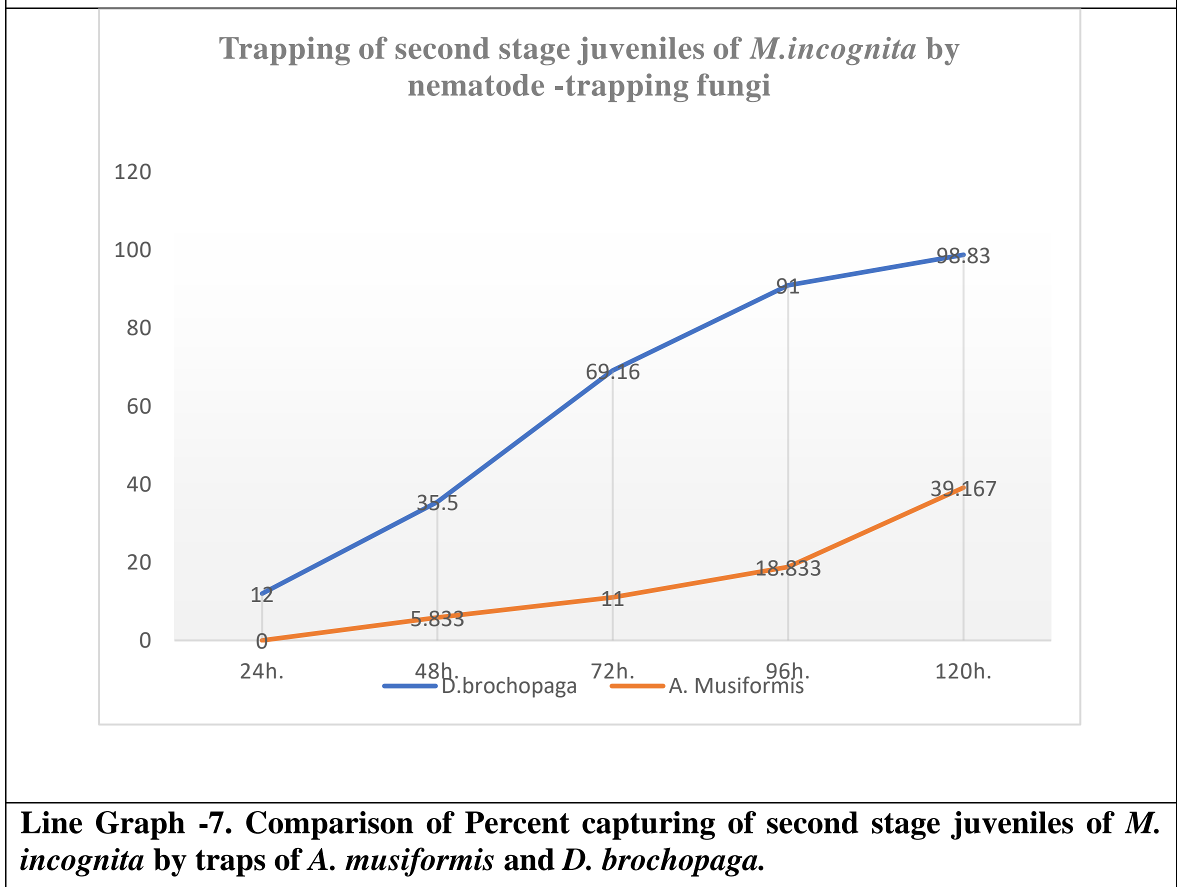
Line Graph -5. Comparison of sporulation of *A. musiformis* and *D. brochopaga* on different media/ substrates.

Table-6 Assessment of *in vitro* nematode-trapping ability of *Drechsllerella brochopaga* against second stage juvenile of *M. incognita* in corn meal agar medium (0.4%)					
Observations	**Days of observations after inoculation of 200 J_2 of *M. incognita***				
	24h.	**48h.**	**72h.**	**96h.**	**120h.**
Average number of constricting rings	3.33^{a}	12.53^{b}	15.63^{c}	21.33^{d}	23.53^{d}
Trapped juvenile (J_2) of *M. incognita*	12.0^{a}	35.5^{b}	69.16^{c}	91.0^{d}	98.83^{a}
Each data superscript with different letters indicates significant difference of row data at P=0.05					

Table-7 Assessment of *in vitro* nematode-trapping ability of *Arthrobotrys musiformis* against second stage juvenile of *M. incognita* in corn meal agar medium (0.4%).					
Observations	**Days of observations after inoculation of 200J_2 of *M. incognita***				
	24h.	**48h.**	**72h.**	**96h.**	**120h.**
Average number of constricting rings	1.3^{a}	2.53^{b}	4.367^{c}	7.66^{d}	9.367^{e}
Percent trapped juvenile (J_2) of *M. incognita*	0.0^{a}	5.83^{b}	11.0^{c}	18.83^{e}	39.16^{f}
Each data superscript with different letters indicates significant difference of row data at P=0.05					



Line Graph 6- Comparison of trap formation in *A. musiformis* and *D. brochopaga* in response to 200 second stage juveniles of *M. incognita*



Line Graph -7. Comparison of Percent capturing of second stage juveniles of *M. incognita* by traps of *A. musiformis* and *D. brochopaga.*

Table No. 8- Germination, conidial trap formation, and trapped nematodes by conidial traps of *D. brochopaga* in soils of different locations of Banda.

Rhizosphere and fallow soil of BUAT, Banda	Conidial Traps (%)	Conidial traps on Germ tube (%)	Germ tube without traps (%)	Non germinated spores (%)	Trapped Nematodes on agar blocks
Lemon Rhizosphreric soil, BUAT, Banda	87.57±3.80	0.22±0.24	0.42±0.35	11.79±3.89	4
Anar Rhizosphreric soil, BUAT, Banda	75.16±1.67	0.65±0.52	2.28±2.66	21.92±2.54	4
Fig Rhizosphreric soil, BUAT, Banda	74.62±2.79	0.00±0.00	14.14±3.94	11.24±3.59	3
Guava Rhizosphreric soil, BUAT, Banda	65.83±1.64	0.19±0.21	5.95±2.78	28.03±2.59	5
Chick pea Rhizosphreric soil, BUAT, Banda	75.10±2.54	0.11±0.36	17.88±3.58	6.91±2.46	4
Fallow soil, BUAT, Banda	57.95±2.53	0.00±0.00	5.56±1.57	36.49±2.44	0.0
Bael Rhizosphreric soil, BUAT, Banda	76.79±2.04	0.43±0.57	1.63±2.01	21.15±1.54	5
Wheat Rhizosphreric soil, BUAT, Banda	73.85±3.55	1.79±1.80	3.37±3.66	21.00±2.88	4
Soil beneath the Neem Tree	73.69±2.37	0.43±0.54	3.26±2.57	22.62±2.98	7
Mustard Rhizosphreric soil	74.76±2.53	0.92±1.47	3.23±2.91	21.10±3.77	6
C.D. @ 5%	2.227	0.683	2.363	2.494	-

Table No. 9 Germination of *Arthrobotrys musiformis* in soils of different locations of Banda.

Rhizosphreric and fallow soil of BUAT, Banda	**Germination (%)**	**Ungerminated (%)**	**Conidial traps (%)**	**Conidial traps on germ tube (%)**	**Trapped Nematodes on agar blocks**
Lemon Rhizosphreric soil, BUAT, Banda	75.92±2.55	24.07±2.54	-	-	-
Anar Rhizosphreric soil, BUAT, Banda	97.39±1.95	2.60±1.94	-	-	-
Fig Rhizosphreric soil, BUAT, Banda	82.57±3.41	17.42±3.41	-	-	-
Guava Rhizosphreric soil, BUAT, Banda	93.08±2.17	6.91±2.17	-	-	-
Chick pea Rhizosphreric soil, BUAT, Banda	85.07±1.82	14.92±1.81	-	-	-
Fallow soil, BUAT, Banda	85.45±2.12	14.54±2.12	-	-	-
Bael Rhizosphreric soil, BUAT, Banda	95.79±2.04	4.20±2.03	-	-	-
Wheat Rhizosphreric soil, BUAT, Banda	88.65±2.45	11.34±2.45	-	-	-
Soil beneath the Neem Tree	90.83±2.26	9.16±2.25	-	-	-
Mustard Rhizosphreric soil	87.09±2.96	12.90±2.95	-	-	-
C.D. @ 5%	**2.046**	**2.046**	**-**	**-**	**-**

Table-10 Performance test of root-knot disease of Brinjal by application of *A. musiformis* and *D. brochopaga*

Treatments	Plant Height (cm)	Avg. no. of root knots	% Reduction in Root knots	Avg.no. of second stage juveniles *M. incognita*	% Reduction in *M. incognita* (J_2)
A. musiformis+ Meloidogyne incognita	25.16±1.33	237.8±8.1	38.99±1.66	5420.9±441.49	50.76±3.62
D. brochopaga+ Meloidogyne incognita	28.19±1.76	125.3±6.3	67.85±1.59	2366.7±94.12	78.50±0.95
***Meloidogyne incognita* Alone (sick soil)**	16.70±1.75	389.8±7.5		11011.1±516.26	
C.D. @ 5%	**1.580**	**7.184**		**385.017**	

Chapter-V

DISCUSSION

The occurrence of various nematode-trapping fungi namely *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys eudermata, Arthrobotrys cladodes,* and *Stylopege hadra* in culture plates planted with soil and decaying plant material of Banda district indicates that these fungi are found in the soil of Banda which is a true representative of Uttar Pradesh Bundelkhand. Wide distribution of nematode-trapping in variety of habitats such as agricultural, horticultural, 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, respective conidiophores and other morphological structures of nematode-trapping fungi used in present investigation were found similar to *Drechslerella brochopaga* and *Arthrobotrys musiformis* as reported by Dreshsler (1937) and mentioned in the identification key given by Cook and Godfrey (1964). Hence, the two species of nematode-trapping fungi studied in detail during present investigation were identified as *Drechslerella brochopaga* Drechsler and *Arthrobotrys musiformis* Drechsler (Fig.1 and Fig. 3). The abundant production of constricting rings on hyphae of *D. brochopaga* and adhesive network in *A. musiformis* 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 free living and plant parasitic nematodes by *Drechslerella brochopaga* Drechsler and *Arthrobotrys musiformis* Drechsler by forming three celled constricting ring and adhesive nets also indicates that these fungi having nematophagous ability in nature and in different habitats (Fig.2&5).

The slow growth of *D. brochopaga* and fast growth of *A. musiformis* on different substrates/semi synthetic media and their conidia production (Table-4&5; Line Graph-1to5) may be attributed to their nature of the growth and sporulation and the types of nutritional components available in the different media (Ananko and Teplyakova, 2011; Kumar *et al*., 2005). Excellent growth and sporulation of *D. brochopaga* and *A. musifornis* on some of the

grains and brans media may be attributed to the availability of all the necessary nutrients that support the good growth and sporulation. Differential growth and sporulation response of *D. brochopaga* and *A. musiformis* on the same culture media may be attributed to the different nutritional requirement of both the fungi (Table-4&5; Line Graph-1to5). The research on other media/substrates may be useful to increase the growth and sporulation of both fungi for mass production and development of commercial formulation for bio control purposes at farmer's field. Kumar *et al.* (2005) also studied the growth and sporulation of five isolates of *A. dactyloides* on five synthetic media, five grains media and six brans media and reported that the barley grain and pea bran media was found superior for mass culture for *A. dactyloides*.

The formation of average number of constricting rings and adhesive traps in 1.66 mm^2 area (10x objective lens) of hyphal growth of *D. brochopaga* and *A. musiformis* respectively after inoculation of second stage juvenile of *M. incognita* 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 adhesive traps of *A. musiformis* indicates (Table-6&7; Line Graph-6 &7) the higher sensitivity of *D. brochopaga* to the trap inducing substances produced by *M. incognita* in dual culture. Similarly higher per cent of trapping *of M. incognita* by *D. brochopaga* in comparison to *A. musiformis* is attributed to the higher number of traps induced within 24 h. of interface between *D. brochopaga* and *M. incognita* in comparison to interface between *A. musiformis* and *M. incognita* The absence of traps in nematode free culture of *A. musiformis and D. brochopaga* 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 nematophagous than the other nematode trapping fungi forming adhesive hyphal nets. Observation of higher trapping of *M. incognita* by *D. brochopaga* in comparison to *A. musiformis* indicates the possibility of the use of *D. brochopaga* as bio management agent for reduction of plant parasitic nematodes in root knot infested fields.

Inhibition of spore germination of fungi due to soil fungistasis is an established phenomenon occurring in all type of soils environment (Dobbs and Gash, 1965; Lockwood, 1977, Kumar *et al*., 2015) and fungistatic ability of soil varies with the changing of physic-chemical properties of soil (Handelsman and Stabb, 1996; Mondal and Hyakumachi, 1998;

Qian and Johnson, 1987.). In the present study, it was observed that majority of spores of *A. musiformis* germinated in soil by germ tube formation whereas conidia of *D. brochopaga* formed the conidial traps with little to moderate inhibitory effect on spore germination (Table-8, Fig.6). Formation of conidial traps by majority of spores of *D. brochopaga* in response to different soils Banda (Table-8) revealed that the diffusates reached from soils to the agar discs resulted in inhibition of spore germination (6.91-36.49%) and conidial trap formation (57.95-87.57%). Trap formation in nematode-trapping fungi in response to nematode metabolite 'Nemin' and conidial trap formation due to soil fungistasis has been reported by several workers (Pramer and Stoll, 1959; Tarjan 1960; Feder *et al.*, 1960; Feder *et al.* 1963; Monoson *et al.*, 1974; Kumar *et al*., 2015). The results indicates that soil contain enough 'Nemin' due to presence of nematodes which induced trap formation directly on the conidia and conidial germ tubes of *D. brochopaga* in close vicinity of the soil. Formation of conidial traps of *D. brochopaga*, with no further extension without trapping of nematodes is attributed to the strong fungistatic properties soil and nematode-trapping ability of this fungus. Germination of *A. musiformis* and conidial trap forming ability of *D. brochopaga* in close vicinity to agricultural soil indicates that these fungi are adoptive in the fungistatic environment of soil of Banda. However, Mankau (1962) reported that the conidial trap formation of nematode-trapping fungi in fungistatic conditions of soil may be a response to antagonism caused by soil microbes. Further, Persmark and Nordbring-Hertz (1997) stated that conidial trap formation in nematode trapping fungi is a response of competition for nutrients with other microorganisms. Formation of frequent conidial traps by *D. brochopaga* in vicinity of soil of Banda without further extension of hyphae showed its ability to survive in the soil in nematophagous state. Conidial trap formation in some species of nematode-trapping fungi were also reported in response to cow dung (Dackmam and Nordbring-Hertz, 1992), soil fungistatsis (Mankau, 1962; Kumar *et al*., 2015), rhizosphere soil or soil extracts (Persmark and Nordbring- Hertz, 1997), and conidial trap formation was believed to be a response to nutrient deprivation due to strong nutrient competition between soil microorganisms. Barron (1992) stated that formation of trapping organs in nematode-trapping fungi give the fungi a competitive advantage over many other fungal saprophytes growing in soil environments that are characterized by low levels of nitrogen. Persmark and Nordbring-Hertz (1997) reported that *Arthrobotrys dactyloides* and *Monacrosporium gephyropagum* are the fungi most capable of forming a conidial traps. Dackman and Nordbring-Hertz (1992) reported the formation of conidial traps as survival structure of

nematode-trapping fungi in intense competitions in soil. Kumar *et al.,* (2015) worked on the carnivorous ability of *D.dactyloides* in fungistatic environment of soil and reported the frequent formation of conidial traps and trapping of nematodes by *D. dactyloides* in majority of soil of India.

Application of mass culture of *D. brochopaga* and *A. musiformis* significantly reduced the number of root-knots and second stage juveniles in brinjal in pot condition. However, maximum reduction in number of root-knots and J_2 of *M. incognita* was found with the application of *D. brochopaga* followed by *A. musiformis* (Table-10). The higher reduction in the root-knot formation and second stage juveniles (J_2) in the roots of brinjal may be attributed to the reduction in the population of seconds stage juveniles in soil by nematode-trapping activities of mass culture of *A. musiformis* and *D. brochopaga* applied in soil. It appears that *D. brochopaga* and *A. musiformis* trapped and kill the second stage juveniles of *M. incognita* and reduce the population level in soil and thus restricted infection of root up to certain level. The higher bio-management potential of *D. brochopaga* in comparison to *A. musiformis* may be attributed to its ability to form conidial traps and trapping of nematodes in soil. It appears that higher cfu of *D. brochopaga* per kg of soil ($4.8x10^6$) formed much more tarps on the conidia and mycelium in the soil resulted in the maximum trapping of *M. incognita* in soil which resulted in the less root knot infection. Singh *et al*. (2007) also reported the good control of root-knot disease of rice by application of *D. brochopaga* @ 4600 cfu per gram of soil. Singh *et al.* (2012 a,b,c) and Singh *et al.* (2013) also reported the nematode trapping fungi reduce the infection of root-knot nematode in rice and tomato.

Chapter-VI

SUMMARY AND CONCLUSION

Meloidogyne incognita is one of infamous species of plant-pathogenic nematode that cause root- knot disease in many agricultural and horticultural crops. Due to the environmental and human health concern related with the use of nematicides, the application of nematicides has been restricted. Therefore, there is an urgent need to develop alternative, environmentally–friendly, safe and effective management strategies for management of plant parasitic nematodes. In this context, nematode-trapping fungi that capture and kill the nematodes were investigated to explore their potential use as bio control agent of root-knot nematode in brinjal.

During the search of nematode-trapping fungi in the complex soil environment of Banda district of Uttar Pradesh, five species of fungi namely *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys cladodes, Arthrobotrys eudermata,* and *Stylopage hadra* were isolated and identified with the use of relevant literature. Presence of a variety of species of nematode-trapping fungi in the soil of Banda indicates that soil of Bundelkhand region also harbours the species of nematophagous fungi. Out of the five species of isolated nematode-trapping fungi from agricultural and horticultural soil of Banda district of Uttar Pradesh, two species namely *Drechslerella brochopaga* Drechsler forming three celled constricting rings and *Arthrobotrys musiformis* Drechsler forming adhesive network for capturing and killing of nematodes were studied in detail with special emphasis on screening of various media and substrates for growth and sporulation, their *In vitro* nematode-trapping ability, adoptability in soil of Banda and their bio-management potential against *M. incognita* in brinjal.

Potato Dextrose agar media and ten grains and brans media were studied for growth and sporulation of *Drechslerella brochopaga* and *A. musiformis.* Maximum growth of *D. brochopaga* was found on Mungbean grain agar followed by sorghum grain agar, chickpea grain agar, corn meal agar, lentil grain agar, soybean grain agar, wheat grain agar, PDA, wheat bran agar and barley grain agar. The lowest growth of *D. brochopaga* was observed on mix bran agar (bran of chickpea, wheat, and pearl millet) medium. Out of the 11 media/substrates tested for sporulation of *D. brochopaga*, maximum sporulation was observed in corn meal agar medium followed by soybean grain, mix bran, sorghum grain,

barley grain, wheat grain, wheat bran, moong grain, chickpea grain agar, and PDA. Maximum colony growth of *Arthrobotrys musiformis* were observed on lentil grain agar medium and mix bran followed by wheat bran, soybean grain agar medium, barley grain agar medium, corn meal agar medium, sorghum grain agar medium, chickpea grain agar medium, PDA, wheat grain agar medium and mung bean grain agar medium. Maximum sporulation of *A. musiformis* was found on lentil grain agar medium followed by mix bran agar medium, chickpea grain agar medium, sorghum grain agar medium, wheat bran agar medium, corn meal agar medium, barley grain agar medium, soybean grain agar medium, wheat grain agar medium, mung bean grain agar medium, and PDA.

In vitro nematophagous potential of *D. brochopaga* and *A. musiformis* was studied by inoculation of 200 juveniles of *M. incognita* in pure culture in CMA medium (0.4%). Trap formation was observed within 48 h. of nematode inoculation in both the fungi, however, the induction of number of constricting rings of *D. brochopaga* was greater than the hyphal nets of *A. musiformis* 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. *D. brochopaga* captured almost all (98.83%) the nematodes within 120 h. of nematode inoculation. In contrast, lower number of adhesive trap formation and trapping of nematodes was observed in *A. musiformis* which trapped and killed 39.16% juveniles of *M. incognita* within 120 h. of nematode and fungus interaction.

D. brochopaga and *A. musiformis* were studied for their potential to grow in the complex agricultural and horticultural soil of Banda. It was found that *D. brochopaga* and *A. musiformis* grow and proliferate well in agricultural soil with little to higher fungistatic effect. Fungistatic effect caused 6.90-36.48 % inhibition in conidial germination of *D. brochopaga* whereas soil fungistasis caused 2.60-24.07 inhibition in conidia germination of *A. musiformis*. This indicates that soil of this region has various ability of fungistatic properties. Irrespective of various soils, conidia of *D. brochopaga* germinated by formation of conidial traps (57.95-87.57) or on short germ tube (0.42-1.79) and captured the nearby nematodes. The ger tube of *D. brochopaga* without traps ranged between 0.42 to 17.88 %. *A. musiformis* germinated by means of germ tube (75.92-97.39) in response of fungistatic effect of various soils of Banda. No trap formation was observed on the germinated spores of *A. musiformis* within 48 h. of observations. Formation of conidial traps and traps on germ tubes of *D. brochopaga* indicates that this fungus switch over to nematophagous phase

immediately after germination in close vicinity to soil. Trapping of killing of soil nematodes by conidial traps of *D. brochopaga* indicates that this fungus parasitizes the nematodes and proliferate in the soil for reduction of nematode-population in nature.

A. musiformis and D. brochopaga were grown on sorghum grains for mass production for application into *M. incognita* infested soil. Grain based formulation of *A. musiformis and D. brochopaga* were applied @ 4.8 x10^6 colony forming units per/g. soil in the root-knot infested soil having 2000 second stage juveniles of *M. incognita* before sowing to manage the root-knot disease in brinjal. Application of nematode-trapping fungi in root-knot infested soil caused reduction in root-knot infection in brinjal roots in pot experiment. Higher reduction in number of root-knot (67.85%) and number of second stage juveniles of *M. incognita* (78.50%) was found in plants grown in soil treated with formulation of *D. brochopaga* in comparison to the plants grown in *M. incognita* infested soil. *A. musiformis* was found to reduce lower percent of root-knots (38.99%) and number of second stage juveniles of *M. incognita* (50.76%).

On the basis of present research on ''Studies on soil adaptability and bio-control potential of *Drechslerella brochopaga* and *Arthrobotrys musiformis* against *Meloidogyne incognita* on brinjal (*Solanum melongena* L).'' following conclusions were made:

- Presence of *Drechslerella brochopaga, Arthrobotrys musiformis, Arthrobotrys cladodes, Arthrobotrys eudermata* and *Stylopage hadra* in soil of Banda district indicates that nematode-trapping fungi are essential component of the soil of Bundelkhand region.
- *D. brochopaga* is more nematophagous due to abundant trap formation and trapping and killing of *M. incognita* in comparison to *A. musiformis*.
- Corn meal agar medium and soybean agar medium was found good for both growth and sporulation of *D. brochopaga*.
- Lentil grain agar medium was found best culture medium for growth and sporulation of *A. musiformis* followed by mix bran and chickpea grain.
- *A. musiformis* is adoptive in most of the soil because of more than 75% germination in various rhizospheric and field soil without formation of conidial traps. The *D. brochopaga* is adoptive in parasitic phase in most of the soil of Banda due to frequent formation of conidial traps and trapping of nearby soil nematodes.

- *D. brochopaga* switch over to parasitic phase in vicinity to soil due to formation of frequent conidial traps and further grow in the soil after capturing and killing of nematodes.
- *D. brochopaga* was found to have potential for controlling the root-knot disease of brinjal by 67.85 % reduction in number of root knots followed by *A. musiformis* which control the root-knot by 38.99%.
- Frequent conidial trap formation, trapping of soil nematodes, and good bio management ability of *D. brochopaga* indicates that this fungus is adoptive in soil and could be delivered in nematode infested soil in form of spore-based formulation for management of root-knot of brinjal.

Chapter-VII

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VITAE

Particular	Details
Name	Radha Krishan
Date of Birth	28/04/1998
Sex	Male
Place of birth	Vill-Udhampur, Post- Kumhawar, District –Etawah (206253)
Address	Vill-Udhampur, Post- Kumhawar, District –Etawah (206253)
Nationality	Indian
E-mail	radhakrishna28041998 @gmail.com
Contact Number	7599229450

ACADEMIC QUALIFICATION

S. No.	Qualification profile	Institute name	Passing year	%
1	HIGH SCHOOL	UP Board Allahabad	2013	75.6
2	INTERMEDIATE	UP Board Allahabad	2015	74.1
3	B.Sc. (Ag.) Hons.	Chandra Shekhar Azad University of Agriculture & Technology, Kanpur	2020	75.3
4	M.Sc. (Ag.) Plant Pathology	BUA&T, Banda	2022	----

Place:

Date