Morphological and molecular characterization of nematophagous fungi *Stylopage hadra* and *Acaulopage pectospora*

काशी हिन्दू विश्वविद्यालय

BANARAS HINDU UNIVERSITY

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of science (Agriculture)

in

Plant Pathology

Submitted by

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Dedicated

to

My Beloved Parents

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CERTIFICATE

To,

The Registrar (Academic)

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Through: The Head, Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi – 221005.

Dear Sir,

I have great pleasure in forwarding the thesis entitled**, Morphological and molecular characterization of nematophagous fungi *Stylopage hadra* and *Acaulopage pectospora*** submitted by **Mr. Sparsh Tiwari, I.D. No. 18412MPP014** in Partial fulfilment of the requirements for the degree of **Master of Science (Agriculture) in Plant Pathology**, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire work was presented in this thesis, was planned and carried out solely by the candidate under my supervision. To the best of my knowledge, the data presented in the thesis are genuine and original. Sources of materials and help obtained from the others are duly acknowledged. I also certify that neither the thesis nor its part there of has been previously submitted by him for a degree of any university.

Thanking you,

Yours faithfully

Forwarded by

Head **Dr. R.K. Singh**

(Supervisor)

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Place: ***Varanasi*** (***Sparsh Tiwari***)

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ABBREVIATIONS

%	Percent
@	At the rate
°C	Degree Celsius
µg	Microgram
B.C.	Before Christ
bp	Base pair
Cl^-	Chloride ion
cm	Centimetre
DAPI	4′,6-diamidino-2-phenylindole
DEPC	Diethyl pyro carbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DSW	Distilled sterilized water
EDTA	Ethylene diamine tetraacetic acid
ET	Ethylene
et al.	And other
EtBr	Ethidium Bromide
FAO	Food and Agricultural Organization
FW	Fresh weight
G	Gram
H^+	Hydrogen ion
H_2O_2	Hydrogen peroxide
Ha	Hectare
HCl	Hydrochloric acid
K^+	Potassium ion
Kg	Kilogram
m	Meter
M	Molar
Mg	Milligram

Mha	Million hectares
Mt	Million tonnes
Ml	Millilitre
mm	Millimetre
N	Normality
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
nm	Nanometer
OH	Hydroxyl ion
PBS	Phosphate Buffer Saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
WA	Water agar
BLAST	Basic Local Alignment Search Tool
RNA	Ribonucleic acid
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
spp.	Species
T_m	Melting temperature
UV	Ultraviolet
V/V	Volume per volume
viz.	Vide licit/namely
µl	Microlitre
µm	Micrometer

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CHAPTER 1

INTRODUCTION

Nematodes are diverse, microscopic, multicellular animals comprising free-living to plant parasitic nematodes. They can parasitize a good range of host including plant species and are major biotic factor limiting crop productivity and ultimately causing crop production, resulting in significant loss worldwide. In a survey carried out for maximum losses by plant parasitic nemtodes it was found that Root-knot nematodes (*Meloidogyne*) cyst nematodes (*Heterodera* and *Globodera*) and lesion nematodes (*Pratylenchus*) ranked at the top ten most economically and scientifically important pathogen species causing maximum crop loss (Jones *et al.*,2013).

Being ubiquitious in nature, they are associated with nearly every commercially important agricultural crop, thus representing significant constraint on global food security. Crop losses due to nematodes are difficult to estimate accurately. Plant-parasitic nematodes cause 21.3% crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually (NAS letters, 2020).

Plant parasitic nematodes not only cause damage individually but also form disease-complexes with other micro-organism and increase the crop loss. The symptoms of nematode damage, resembles with the symptoms of other pathogens and abiotic stresses like water and mineral deficiency. Nematode diseases are difficult to diagnose and manage because of their hidden nature and hence, more often overlooked.

Limitations on the utilization of chemical pesticides with increasing demands in organic agriculture and concerns for environmental welfare have brought increasing interest in studies on alternative methods of nematode control. Among several strategies of management biological control of nematode is one of the eco-friendly methods. Under biological control use of nematophagous fungi i.e nematode killing fungi are found potential option for the nematode management (Bernard *et al.,* 2017).

Term "nematophagous fungi" is employed to explain a various group of organisms with the ability to infect and parasitize nematodes for the advantage of nutrients. The first description of their nematophagous habit came within the late 1800 by Fresenius, (1852) and Zopf, (1888) Drechsler, (1937) provided voluminous and detailed work describing in greater detail the predatory activity of several species of these fungi. The reason for the continuing interest in these fungi is, in part, their potential as biocontrol agents against plant- and animal-parasitic nematodes. One reason for the continued fascination in nematophagous fungi is it's remarkable morphological adaptations and fascinating capturing of nematodes. Nematophagous fungi are found in most fungal taxa: Ascomycetes (and their hyphomycete anamorphs), Basidiomycetes, Zygomycetes, Chytridiomycetes and Oomycetes. (Ciancio and Mukerji, 2007). Therefore it appears that the nematophagous character evolved independently within the different fungal taxonomic groups. Barron (1992) suggested that the nematophagous habit might have been evolved from lignolytic and cellulolytic fungi, as an adaptation for competition of nutrients in soil.

The commonly found nematophagous fungi are nematode-trapping fungi, they capture nematodes with the help of hyphal trapping devices of varied shapes and sizes, e.g. adhesive three dimensional nets, adhesive knobs, non-adhesive constricting rings. Depending on their mode of parasitism, the nematophagous fungi are categorized into four groups: (i) nematode-trapping (formerly called predacious or predatory fungi), (ii) endoparasitic, (iii) egg- and female-parasitic and (iv) toxin-producing fungi (Jansson and Lopez-Llorca, 2001). Over 200 species of fungi belonging to different taxa catch free-living nematodes in the soil using traps produced by the fungal mycelium that adhere to the worm, then penetrate, kill, and digest the tissue of the nematode.

A couple of nematode-trappers capture nematodes without visible traps in an adhesive substance formed on their hyphae, e.g. *Stylopage* and *Acaulopage* species which are predominantly known as amoebaphgous fungi i.e. capturing and killing amoebae. The species of both fungus *Stylopage* and *Acaulopage* belongs to the class Zygomycetes (Winter 1881), which characteristically formed zygosporangia in which resistant spherical spores are formed during sexual reproduction. This class also

includes many other Nematopahgous as well as amoebaphagous fungi such as *Cochlonema, Endocochlus, Bdellospora, Stylopage, Acaulopage* (Amoebaphagous) and *Euryancale* (internal parasite of nematode).

The zoopgales were recently placed in the newly described Zoopagomycota a lineage formerly belonging to zygomycota which primarily associated with other microbes and animals (Spatafora et. al. 2016). The zoopagales contains five families delineated using ecology and morphology of conidia and conidiophores and method of penetrating the prey (Kirk et. al. 2010). However, because zoopagalean taxa are largely uncultured and molecular data are scarce and mostly reported for amoebhagous than its nematophagous potential. There was need to study the nematode trapping potential of *Stylopage* and *Acaulopage* which were reported obligate in nature and required some prey to grow and sporulation as a possible biocontrol for the management of root knot nematodes. In view of this fungus *Stylopage* and *Acaulopage* closely related genera were undertaken for the present study with following objectives.

1. Isolation and purification of *Stylopage hadra* and *Acaulopage pectospora*
2. Morphological and molecular characterization of *Stylopage hadra* and *Acaulopage pectospora*
3. Effect to temperature and media on the growth of *Stylopage hadra* and *Acaulopage pectospora*
4. *In vitro* predacity test of *Stylopage hadra* and *Acaulopage pectospora* against second stage juveniles

CHAPTER 2

REVIEW OF LITERATURE

2.1 History and Importance

Nematophagous fungi are microfungi that can trap, kill and digest nematodes. They parasitize nematodes by using special mycelial structures, known as traps, or spores to capture nematodes or hyphal tips to attack nematode eggs and cysts before they penetrate into nematode cuticle followed by invasion and digestion. Nematophagous Fungi are potential candidates for biological control of plant parasitic nematodes, and thus serves as an important constituent in integrated pest management programs.

The first nematophagous fungus was recognized and characterized by the great mycologist Fresenius in 1852 and named as *Arthrobotrys oligospora*. He was able to observe the spores and mycelium of *Arthrobotrys oligospora* with great accuracy but was unaware about the predacious nature of fungus which was grown to supplement fungual growth by capturing nematodes i.e. generally found as saprophytic but switch over to parasitic stage by forming trapping structure in presence of nematodes. Woronin, (1870) for the first time observed the hyphal bail formation of this fungus, but he did not know the function of such bails. Zopf, (1888) was the first to record the predacious behaviour of *A. oligospora*. The observations of Zopf where repeated nearly 50 years later by Drechsler. Drechsler with his voluminous provided voluminous work over the predacious fungi. He not only corrected Zopf's original account but also described many other predacius fungi and their trapping devices which were impressively reviewed by Duddington (1962).

The Genus *Stylopage* was first described by Charles Drechsler in 1935, with the discovery and growth of three new species *S. haploe, S. araea,* and *S. lepte* on media that had been grown from decaying vegetable matter found in temperate woodlands followed by identification of fifteen species of the Stylopage and 5 species of *Acaulopage* i.e. *A. tetracerros, A. ceratospora, A. macrospora, A. rhapidospora* and *A. rhincospora.*

Among more than 26,000 known species of nematodes, 8000 are parasites of vertebrates (Hugot *et al*. 2001), whereas 4100 are parasites of plants, mostly soil-borne root pathogens (Nicol *et al*. 2011 Among more than 26,000 known species of nematodes, 8000 are parasites of vertebrates (Hugot *et al.* 2001), whereas 4100 are parasites of plants, mostly soil-borne root pathogens (Nicol *et al*. 2011 Among more than 26,000 known species of nematodes, 8000 are parasites of vertebrates (Hugot *et al*. 2001), whereas 4100 are parasites of plants, mostly soil-borne root pathogens (Nicol *et al*. 2011) Among more than 26,000 known species of nematodes, 8000 are parasites of vertebrates (Hugot *et al*. 2001), whereas 4100 are parasites of plants, mostly soil-borne root pathogens (Nicol *et al*. 2011) Nematode damage in crops is non-specific and causes a range of symptoms from mild to severe, such as wilting, stunting, reduced vigour, nutrient deficiency, root lesions, re- duced flowering, fruit loss, poor yield, and even death. Mild symptoms may be overlooked, and even the severe symptoms can be misdiagnosed (Nicol *et al.* 2011) Nematode damage in crops is non-specific and causes a range of symptoms from mild to severe, such as wilting, stunting, reduced vigour, nutrient deficiency, root lesions, reduced flowering, fruit loss, poor yield, and even death. Mild symptoms may be overlooked, and even the severe symptoms can be misdiagnosed (Nicol *et al.* 2011) Nematode damage in crops is non-specific and causes a range of symptoms from mild to severe, such as wilting, stunting, reduced vigour, nutrient deficiency, root lesions, reduced flowering, fruit loss, poor yield, and even death. Mild symptoms may be overlooked, and even the severe symptoms can be misdiagnosed (Nicol *et al.* 2011)

2.2 Taxonomy of *Stylopage* and *Acaulopage*

Nematophagous fungi are present in all major taxonomic groups of fungi, including lower (oomycetes, chytridiomycetes and zygomycetets) as well as higher fungi such as the ascomycetes and basidiomycetes. Yu *et.al*, (2014) found nematophagous fungi comprise more than 700 described species from several phyla, such as the Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota. They have the ability to attack living nematodes (juveniles, adults and eggs) and use them as nutrients. Therefore, it appears that the nematophagous habit evolved independently in the different fungal taxonomic groups.

Drechsler, (1938) defined the zoopagaceae as a family of class Zygomycetes consisting entirely of predacious species. Most of them attack terricolous amoebae, or capture even nematodes. Drechsler earlier placed *Stylopage* in Phycomycetes after identification of new *Stylopage* species, he placed them in new genus within the, Zoopagaceae of Zygomycetes. Identification and description of other *Stylopage* species were differentiated and based on conidial morphology and their predatory behaviour. *Stylopage* has been described as both a predator and an obligate parasite exhibiting predatory behaviour towards either amoebae or nematodes. The predacious zygomycetes, along with many parasitic taxa, were placed in Zoopagales (Benny et al. 2016) and concluded that these cannot be cultured in the absence of a prey population.

Winter,(1881) stated that *Stylopage* and *Acaulopage* belongs to the class Zygomycetes forming Zygospores, characteristically formed by the members of this clade, in which resistant spherical spores i.e. zygospores are formed due to sexual reprodcution. This class has many nematophagous as well as amoebaphagous fungi such as *Cochlonema, Endocochlus, Bdellospora* (Amoebaphagous) and *Euryancale* (internal parasite in nematode)

Spatafora *et al*., (2016) reclassified the orders formerly belonging to Zygomycota, including Zoopagaceae, *Stylopage*, *Acaulopage* and other zoopagales placed within the phylum Zoopagomycotina, under the new phylum zoopagomycota, a lineage formerly belonging to Zygomycota that primarily associates with other microbes and animals The zoopagales have been since proven as a monophyletic order within the zoopagales. *Stylopage* forms a sister clade with genus *Acaulopage* widely studied as an amoebaphagous fungi, with only few reported cases of nematode predation.

However at molecular level, more recent phylogenetic tree reconstructions based on 18s rRNA phylogeny described by Davis *et.al*., contend that *Stylopage* itself is not monophyletic, and that *Stylopage hadra* forms a sister clade with *Zoophagus* species, which suggests that morphological similarities i.e conidial morphology is not sufficient enough to determine monophyly within the genus. They generated 18S sequences for *Acaulopage, Stylopage, Zoophagus* and concluded that 93% similarity

of *A. tetraceros* with gene bank assession number JQ288098 while 98% similarity of sequence of *Zoophagus insidans* with Gene Bank (AB016009) while sequence of *S. hadra* was 99% similar to the sequence in Gene Bank (EF546661)

Classification of *Stylopage* and *Acaulopage*:

The fungus *Stylopage* and *Acaulopage* were first identified and thus reported by Charles Drechsler in 1935. He identified 3 species of *Stylopage*, viz. *S. haploe, S. araea* and *S. lepte* on media that had been grown from decaying vegetable matter found in temperate woodlands. Total 15 species of *Stylopage* had been reported, among which many behave as predator and parasite as well upon amoebae and nematode whereas the species of *Acaulopage* i.e. *A. tetracerros, A. ceratospora, A. macrospora, A. rhapidospora* and *A.rhincospora* were recorded by him. They were mostly recovered from soil or leaf litter when placed on agar in petri dishes. Dayal, (1973) described twenty seven species of *Acaulopage*. All of them were parasitic to amoebae except *Acaulopage pectospora* which was parasitic to nematodes. *Acaulopage tetraceros* is the most commonly reported species. They had undergone a number of taxonomic modifications and currently placed as given in Table 1.1

Table 2.1: Classifiaction of *Acaulopage* and *Stylopage* species

Kingdom : Fungi	Kingdom: Fungi
Phylum : Zoopagomycota	Phylum : Zoopagomycota
Class : Zoopagomycotina	Class : Zoopagomycotina
Order : Zoopagales	Order : Zoopagales
Family : Zoopagaceae	Family : Zoopagaceae
Genus : *Acaulopage*	Genus : *Stylopage*
Binomial name: ***Acaulopage* spp**. (Drechsler, 1935)	Binomial name: ***Stylopage* spp.** (Drechsler,1935)

2.2 (a) Morphological Characteristics

Kirk *et al*. (2010), founded that the genera in Zoopagales were classified on the basis of conidium and conidiophore morphology, whereas species were delineated based on their ecology, conidium or conidiophore morphology, trapping structures formed and infection mechanisms.

Stylopage was characterized by effused mycelium with continuous, sparsely growing, dichotomously branched, aseptate vegetative hyaline hyphae, and prey by means of adhesive material. In *S. hadra*, the adhesive substance on the vegetative hyphae appears as golden-yellow colour. The hyphae then penetrates integument of prey by means of a lateral branch, produced haustorium, or an internal mycelium, which utilizes the prey content and bear a single conidium at its apex, followed by repeated elongation with several conidia produced successively . The size and shape of conidia, number of conidiophore, dimensions of hyphae and presence or absence of zygospores were the main criteria of differentiation of *Stylopage* species (Table 1.2). The genus *Stylopage* differs from its sister clade genera *Acaulopage*, by it's production of aseptate conidia on long, erect conidiophores (Table 1.3).

Table2.2: **Morphological variation in *Stylopage* spp.**

S. no	Species	Predation	Vegetative hyphal morphology	Conidia & conidiophore morphology	Zygospore morphology	Reference
1	*S. anomala*	Amoebae	Hyaline, sparsely branched, 3.0-6.0 µm wide. Growths observed at attached points of amoebae to the mycelium.	Conidiophores hyaline, erect, 95-160 µm long, each producing up to 5 conidia. Conidia obvoid, 16-39 µm long and 6.0-14.5 µm wide. Conidia bear a round protuberance at their apical end filled with an adhesive substance; this substance attaches two or more conidia together	Zygospores unknown.	Wood, (1983);Blackwell *et.al*.,(1991).
2	*S. araea*	Amoebae	Hyaline, sparse, 0.8-1.3 µm wide.	Conidiophore hyaline, erect, comparatively slender and unbranched, 150-225 µm long, each bearing one conidium. Conidia drop-shaped with a pointed basal end and rounded apical end, 10-22 µm long and 5.4-7 µm wide.	Zygospores yellow with wart-like bumps,(9-12 µm in diameter.)	Jones, (1962); Drechsler, (1939); Drechsler, (1935) Peach *et. al*.(1955);Michel *et al*.(2019);
3	*S. cephalote*	Amoebae	Colourless, sparse, 1.2-1.8 µm wide.	Conidiophore hyaline, erect, 45-75 µm long, with 4-9 conidia borne radially at the end of each conidiophore; conidia cylindrical, rounded at the distal end and pointed at the base, 14-	Zygospore (7-9 µm in diameter), yellowish, round wart-like bumps.	Drechsler, (1938)

				25 µmlong and 1.8-2.5 µm wide.		
4	*S. cymosa*	Amoebae	Hyaline, sparsly branched, 1-2 µm width.	Conidiophore erect, up to 150 µm in height, each bearing up to 8 conidia successively; conidia are hyaline and rectangular-ellipsoidal, 12-21 µm long, 6-10 µm wide.	Zygospores unknown.	Duddington, (1953)
5	*S. grandis*	Nematodes	Sparsely branched hyaline vegetative hyphae 5 µm wide.	Conidiophore erect, 300-500 µm long, bearing maximum 2 conidia; conidia obovoid, conidia size is 27-61 µm long and 13-26 µm wide.	Zygospores unknown.	Duddington, (1955)
6	*S. hadra*	Nematodes	Colourless, sparsely branched, 3.5-5.5 µm wide. Yellow-orange adhesive protuberances up to 15 µm wide upon contact with nematodes.	Conidiophore tapering, 200-400 µm long, 2.5 µm (at tip) to 4.5 µm (at base) wide, can bear up to 4 conidia successively; conidia obovoid, 20-45 µm long and 13-23 µm wide.	Zygospores unknown.	Drechsler, (1935)
7	*S. haploe*	Amoebae	Colourless, sparsely branched, 1-1.7 µm wide.	Erect, tapering conidiophores, 25-40 µm long and 5.4-7 µm wide, each bearing a single conidium; conidia fusoid and rounded at ends, 15-25 µm long and 2.2-2.7 µm wide.	Zygospores up to 10 µm in diameter, covered in small wart-like bumps.	Drechsler, (1935).

8	*S. leiohypha*	Nematodes	Colourless, sparsely branched, 2-3 µm wide.	Conidiophore colourless, erect, 125-300 µm long, tapering (up to 3.5 µm wide at the base and up to 1.4 µm wide at the apical tip), and each producing up to 4 conidia successively. Conidia, colourless, drop-shaped, with a pointed basal end and rounded apical end, 20-35 µm long and 7-18 µm wide.	Zygospores unknown.	Drechsler, (1936)
9	*S. lepte*	Amoebae	Colourless, sparsely branched, 0.6-1 µm wide.	Erect, colourless conidiophores, 25-100 µm long and 0.7-0.9 µm wide, each bearing up to 6 conidia successively; conidia are drop-shaped, with one pointed and one rounded end, 12-19 µm long and 1.9.2-7 µm wide. Conidia can produce haustoria directly.	Zygospores 4.5-6.5 µm in diameter, colourless or yellowish, with 10-15 wart-like bumps. Zygospores can form through lateral conjugation.	Drechsler, (1935).
10	*S. minutula*	Amoebae	Sparse, threadlike, continuous vegetative hyphae, 0.6-0.9 µm wide; haustoria terminate after 2 bifurcations within the prey	Erect conidiophore, 40-60 µm long, 0.6-0.9 µm wide, each producing a maximum of 2 conidia successively; conidia ellipsoidal, 7.5-9 µm long and 2.6-3 µm wide.	Zygospores unknown.	Drechsler, (1945)
11	*S. rhabdoides*	Amoebae	Colourless, threadlike with coarse	Conidiophore simple, erect, colorless, 1.4-2 µm wide, and	Zygospores yellow, round, and covered in wart-like	Drechsler, (1947)

			membranes, 1.4-3 µm wide; branching pedicellate haustoria that spread inside prey size of 5-18 µm.	20-50 µm long; conidia elongated and cylindrical with pointed ends, 25-57 µm long and 2.7-5.3 µm wide.	projections, 8-10 µm in diameter, commonly arising from the union of a vegetative hypha and a conidial germ tube.	
12	*S. rhabdospora*	Amoebae	Colourless, 1-1.8 µm wide, with comparatively fewer branches than other *Stylopage* species; haustoria can also be produced directly from conidia.	Conidiophore colourless, erect, and tapering, 20-100 µm high and 0.8-1.5 µm (at the base) to 0.6-1.2 µm (at the apical end) wide; conidia are cylindrical with one pointed end, 25-35 µm long and 2.2-2.8 µm wide.	Zygospores yellowish, 6.5-8.5 µm wide, covered in 10-20 wart-like protuberances. Zygospores can form through lateral conjugation.	Drechsler, (1936;1946)
13	*S. rhicnacra*	Amoebae	Colourless, sparsely branched, 0.9-1.3 µm wide.	Conidiophore colourless, erect, 140-175 µm long, suddenly widening at their end until a septum forms and this widening becomes the conidium; each conidiophore bears one conidium. Conidia fusiform (spindle-shaped), 17-27 µm long and 4.5-6.5 µm wide.	Zygospores unknown.	Drechsler, (1948)
14	*S. rhynchospora*	Amoebae	Colourless, sparse with few branches, 1-1.8 µm wide.	Conidiophore prostrate for a length of 5-20 µm then turn upwards and become erect up to 220 µm, taper, from 3.5 µm near the base to 0.7-0.8 µm at the apical end; each	Zygospore yellowish, 7.5-9 µm in diameter, often fused with the wall of the zygosporangium, covered in large wart-like bumps, of 2 µm	Drechsler, (1939); Jones, (1959)

				conidiophore bears one conidium. Conidia are colourless, ovoid, 29–40.5 µm long and 7-12 µm wide..		
15	*S. scoliospora*	Amoebae.	Colourless, 1-2 µm wide, producing branching pedicellate haustoria inside prey that terminate with 8-10 branches.	Conidiophore 0.8-1.6µm wide and up to 500 µm long, each producing up to 75 conidia successively, with a sudden jagged bend at the point of attachment of each conidium; conidia hyaline, frail, and threadlike, often looking similar to regular hyphae, 20-32 µm long and 1.3-1.9 µm wide.	Zygospores unknown.	Drechsler, (1939)

Acaulopage

Total described species of *Acaulopage* is twenty seven, predating over Amoebae, except *Acaulopage pectospora* which is predatory to nematodes.

Acaulopage pectospora is characterized by moderately branched, colorless mycelium (2.5-5) µm wide normal hyphae, while predacious branches with 10- 25 µm long and 2.5-3-µm wide at different intervals on normal hyphae. The hyphae and branches in many instances ascending distally to terminate on the surface with adhesive knob; about 2.5-µm wide.

The fungal hyphae adhers to a nematode and captures it, then growing out at the tip in producing a clavate or dolioform appressorium 7-25µm long and 5-10µm wide, together with a broadly intruded globose infection-bulb, 4-8µm thick, from which assimilative hyphae, often 2.2-3.2µm wide, are extended length wise through the captive; conidia colorless, solitary, ascending erectly into the air though embedded proximally for a distance of 17-28µm, rather slenderly spindle-shaped, usually 180-240µm long, 7-14µm in greatest width, tapering upward to a diameter of 2µm and downward to a diameter of 1-2µm, sometimes devoid of living protoplasm at the tip for a distance of 7-52µm and at the base for a distance of 1-20µm, connected proximally with a subsurface hypha by an isthmus often 5-12µm long and 1-1.3µm wide.

Although in most predacious members of the Zoopagaceae virtually all portions of the mycelium seem about to be equally capable of holding prey through adhesion, such capability in *Acaulopage pectospora* is restricted to the meagerly differentiated knobs at the tips of many hyphal elements. With respect to their terminal position, the knobs resemble the adhesive cells used in capturing nematodes by clamp-bearing fungi described as *Nemotoctonus haptocladus* and *N. campylosporus*. (Drechsler, 1946, 1954)

Table2.3: Morphological Variation in *Acaulopage* spp.

S.no.	Species	Predation	Vegetative hyphal morphology	Conidia & conidiophore morphology	Zygospore morphology	Reference
1.	*Acaulopage acanthospora*	Amoebae	Hyaline mycelium, branched hyphae 1.5-1.7µm wide	Single,bearing branching with an avg. size 8-10 x 5-6.5µm	Globose zygospore	-
2.	*A. pectospora*	Nematode	moderately branched, hyphae2.3-7.8µm wide.	conidia single, spindle-shaped, imbedded proximally in the substratum, bearing a distal droplet, 180-246µm × 7—14µm	-	Drechsler (1962)
3.	*A. crobylospora*	Amoebae	1.4-1.5 µm wide, moderately branched.	Conidia bearing bush-like branching crest at tip, 10.5—27 x 6.8-1.4- 3µm	-	Drechsler (1947)
4.	*A. dichotoma*	Amoebae	1.3-1.6µm wide,	Conidia occuring singly but branched in regular dichotomous manner, 25-40 x 4.5-7µm	8-13µm diameter, spherical and smooth in early stage and tuberculate at maturity.	Drechsler (1945)
5.	*Acaulopage hystricospora*	Amoebae	Mycelium sparse, sparingly branched; vegetative hyphae color- less, (1µm wide), with	Conidia single. surface material, faintly yellowish, globose or prolate ellipsoidal or oblate ellipsoidal or turbinate, 7.5 to 12.5 x long and 7 to	-	-

			developed haustorium.	14,u wide, 10 to 50 in number all, empty at maturity, mostly finger-shaped, 2 to 6.5 µm long and 0.7 to 0.9 µm wide.		
6.	*Acaulopage lophospora*	Amoebae	mycelium sparse colourless, non-septate hyphae, 1·5-2 µm wide, adhering to minute animals, haustorium bushlike, often consisting of 3 or 4 assimilative branches 5 to 25 ,t long and about 1.2 ,u	conidia colourless, flask-shaped structures, with an empty basal stipe and six to twelve appendages at the distal end. (13-32µm) in length and 8-17µm in greatest width. The appendages were 4·5-12µm long and about 1·5µm wide at the base	-	Drechsler (1946)
7.	*A. longicornis*	Amoebae	-	Conidia bearing appendages in trivaricate manner, 10-17 x 5—10µm	-	Drechsler (1955)
8.	*A. ceratospora*	Amoebae	-	Conidia elongate-ellipsoidal, 20-34 x 4—6 µm, with a lower empty part 2-6 x 0.8-1.2µm and distal tapering empty part 30-70 x 1.3 µm at base and 0,5-0,8 µm at tip.	-	Drechsler (1935)
9.	*A. marantica*	Amoebae	-	Conidia elongate spindle-shaped, 33-52 x 2.4—3.1 µm, with a lower empty distal	-	Drechsler (1939)

				tapering part 15-30 x 0.8-1.3 µm		
10.	*A. rhapidospora*	Amoebae	-	Conidia acircular, straight or curved, 30-40 x 1.2—1.7 µm	-	Drechsler (1935)
11.	*A. ischnospora*	Amoebae	mycelium consist of nonseptate hyphae, 1-2 µm in diameter, and sparingly branched.	conidia filiform, 30-110 µm long and 1-2 µm in diameter. conidia appeared to be completely filled with cytoplasm without the distal empty appendage, stood erect while attached to the mycelium.	Zygospore Unknown	Drechsler (1947)
12.	*A. stenospora*	Amoebae	-	Conidia tapering at both ends, 25-60 x 1.2-1.6 µm	Zygospore unknown	Drechsler (1941)
13.	*A. aristata*	Amoebae	-	Conidia broad, 13.0-32 x 2.0-4.2 µm.	-	Jones (1959)
14.	*A. gyrinodes*	Amoebae	-	Ellipsoidal part 14-20 x 4-6.5 fi, while upper empty tubular part 20-37 x 1.3-1.9µm,	-	Drechsler (1948)
15.	*A. cercospora*	Amoebae	-	Ellipsoidal part 7-15 x 2.2-3.6µm, while upper empty tubular part 6-20 x 0.4-0.8µm	-	Drechsler (1936)
16.	*A. gomphoclada*	Amoebae	mycelium sparse, sparingly branched. vegetative hyphae hyaline, haustoria pedicellate, slightly	Conidia Fusiform part 11-22 x 1.3-1.8µm;, while upper empty part 8-20 x 0.5 µm.	-	Drechsler (1942)

			flexuous, .6 to 1.3 µm wide			
17.	*A. tenuicornis*	Amoebae	scanty mycelium with colorless filamentous hyphae moderately branched 1-1.7 µm wide	Fusiform part 13-21 x 2.8-3.6 µm, while upper empty part 10-22 X 0.8-1.3 µm at base and 0.4—0.8 µm at apex.	-	Drechsler (1959)
18.	*A. baculispora*	Amoebae	mycelium consisted of sparingly branched, non-septate hyphae, 1-2µm in diameter	Conidia 21-22 X 1.8-2.1µm in size.	-	Drechsler (1948)
19.	*A. retusa*	Amoebae	-	Conidia larger than *A. baculispora,* 24—30 x 1.5µm	-	Jones (1959)
20.	*A. macrospora*	Amoebae	-	Conidia 30-70 x 1.6-2.5 µm	-	Drechsler (1935)
21.	*A. rhicnospora*	Amoebae	hyphae 1.2-1.8µm wide but the haustorial branches growing in the captured amoebae were thicker(1.5-2.5µmwide)	Conidia 20-55 x 1.5-2 µm in size.	-	Drechsler (1935)
22.	*A. tetraceros*	Amoebae	Nonseptate hyphae,1-1.6µm wide	Conidia 16-24 x 7-10 µm mostly 4 appendages	8-12µm diameter, immature were	Drechsler (1935)

					spherical and tuberculate at maturity.	
23.	*A. tetraceros var. longa*	Amoebae	Nonseptate hyphae 1-1.6µm wide.	Conidia larger than A. tetraceros, 24-36 x 4.8-8.7 µm	8-12 µm Tuberculate	Jones & Peach (1959)
.24.	*A. dactylophora*	Amoebae	-	Conidia lobed, 7-9 x 9-14µm, bilobate or trilobate with 10-30 appendages.	-	Drechsler (1955)
25.	*A. dasyspora*	Amoebae	-	Protuberances finger-shaped, expanded at tip or bilobate in shape.	-	Drechsler (1955)
26.	*A. trachyspora*	Amoebae	-	Protuberances tapering, unequal in width, 20-90 in number, conidia 12-18 x 11-27µm		Drechsler (1959)
27.	*A. lasiospora*	Amoebae	Mycelium sparse, sparingly branched; vegetative hyphae colorless, 0.9 to 1.4 µm wide. Haustorium with 2 to 15 branches, which vary from 10 to 50 µm in length and from 1 to 1.3µm in width.	Protuberances varying from 25-125 in number, conidia 12-16 x 11-16µm	-	Drechsler (1942)

2.2 (b) Molecular Characterization of *Stylopage* and *Acaulopage* on the basis of 18s rRNA.

Corsaro *et al*. (2018) proved that the position of conidia is insufficient to distinguish genera such as *Stylopage* and *Acaulopage* and thus sequenced four fungal strains belonging to genera *Acaulopage* and *Stylopage* plus one unidentified isolate. He analyzed additional amoebaphagous zoopagales including, for the first time, a *Stylopage* isolate and various environmental sequences. The results analysed and suggested that in the zoopagomycotina, the amoebaphagous taxa have emerged all on a distant branch of the zoopagales after the radiation of the zoophagous and mycoparasititc lineages. The *zoophagus* clade, showed, contrarily with previous studies, as the most basal lineage, suggesting that the ancestral state of the group might have been zooparasitic.

Davis *et al.* (2019) firstly reported the first sequences for the genus *Zoopage* along with additional sequences of *Cocholonema*, *Acaulopage*, and *Zoophagus*, all of which belongs to zoopagales to test the monophyly of genera and species. They used a single-cell approach to generate nuclear 18S rRNA (18S) sequences and concluded that *Zoophagus*, *Zoopage*, and *Acaulopage tetraceros* are not monophyletic. The whole genome amplification was carried out through Multiple Displacement Amplification, (Gawad *et. al* 2016), as it uses Φ29 DNA polymerase which has higher precision than taq DNA Polymerase (Binga *et al*.2008)

Benny *et al. (*2016) founded that predacious zygomycetes, along with many parasitic taxa, are placed in Zoopagales, which cannot be cultured in the absence of nematode. They founded that order zoopagales contains five families, 23 number of genera and over 200 species classified precisely using ecological and morphological behaviour.

Spatafora *et al*. 2016 described that the zoopagales were currently placed in the newly evolved Zoopagomycota, a lineage which previously belonged to Zygomycota.

Corsaro *et al*. (2019) found that *Stylopage araea* strain SA-ET forms close proximity with closely related *A. dichotoma* strains Ad-Rom and Ad-Syc (98.5%) that cluster robustly together in both the 18S and ITS tree. *A. tetraceros* strain At-Blent (Michel *et al.* 2015) as basal lineage well describing the *Acaulopage* clade. The *Cochlonema* clade was found to be the sister clade to *Acaulopage-Stylopage* clade, with ITS-LSU (28S rRNA) supporting close proximity of *Syncephalis* with *Acauopage* clade.

Tanabe *et al*. (2000) reported that the 18S rDNA phylogeny is mostly consistent with traditional tool and techniques for classification schemes of the Fungi. Michel *et al*., (2015) revealed *Acaulopage* strain At-Blent having an 18S rRNA sequence of 1762 bp in length supporting close proximity among all representatives of order zoopagales.

2.3 Trapping structure induced and mechanism

Drechsler (1946, 1954) described that the genus *Stylopage* name refers to the presence of long, rod- like fertile hyphae, as well as its predatory nature by which it capture sprey in a typical fashion. First, amoeba or nematode makes contact at vegetative hyphae, which have an adhesive substance produced by the fungus at the point of contact. Once the prey organism has been immobilized, a haustorium is produced by the fungus which penetrate its cell membrane and branched inside the organism. Once the internal organs and nutrients of the prey organism have been consumed than the fungus sequentially erect septa within haustoria .This leaves only the outer membrane of the prey organism, which remains attached to the point of contact on the hyphae, even after the haustoria have withdrawn.

Das-Gupta, S. N (1966) described that although in most predacious members of the zoopagaceae virtually all portions of the mycelium seem about equally capable of holding prey through adhesion, such capability in *Acaulopage pectospora* is restricted to the meagerly differentiated knobs at the tips of many hyphal elements..

They described that in *Acaulopage pectospora* fungal hyphae adhers to a nematode and captures it, then growing out at the tip in producing a clavate or dolio

form appressorium 7-25µm long and 5-10µm wide, together with a broadly intruded globose infection-bulb, 4-8µm thick, from which assimilative hyphae, often 2.2-3.2µm wide, are extended lengthwise conidia hyaline, solitary, ascending erectly imbedded proximally for a distance of 17-28µm slenderly spindle-shaped, of 180-240µm long, 7-14µm in greatest width, tapering upward to a diameter of 2µm and downward to a diameter of 1-2µm, sometimes devoid of protoplasm at the tip for a distance of 7-52µm and at the base for a distance of 1-20µm, connected proximally with a subsurface hypha by an isthmus often 5-12µm long and 1-1.3µm wide.

A. pectospora parasitizes the prey by it's axial hyphae on which they are borne. After the capture of prey, when it becomes motionless, assimilative hyphae equal in width to the external mycelium grows from the infection-bulb lengthwise through the fleshy interior. The hyphae not visible in the initial phase of infection but are clearly visible after the content of prey have been absorbed by them. *A. pectospora* sometimes germinate still being in an erect posture by putting a short germ tube laterally or obliquely adjacent to its narrow base. Often such germ tube forms ancurved adhesive knob. Conidia that have toppled may germinate by extending predacious germ hypha from it's distal end and another from it's basal end.

2.4 Nematodes management

The primary groups of nematodes *viz*., root-knot, burrowing, cyst, lesion, foliar, and reniform nematodes are known to cause problems in the tropical and subtropical area. While they incur severe crop losses, few nematodes such as pin, spiral and lance nematodes occur abundantly causing moderate damage to the crops. Root knot nematodes is one among the top most plant pathogen distressing world's food production. They are polyphagous in nature and belong to the genus *Meloidogyne*. These nematodes feed on roots of the plant and mature inside them. Their feeding produces galls by enlarging the roots and infestation severely reduces the quality and yield of the crop. They could not persist for a longer period without a host plant except in the egg stage in small numbers.

Sasser and Freckman (1987) reported that crop production losses due to nematodes in the tropical and sub-tropical climates and developed countries were estimated at 14.6% and 8.8% respectively. Jain *et al.* (2007) assessed the annual crop losses due to important plant parasitic nematodes in India to be about Rs. 242.1 billion. Plant parasitic nematodes interact with other soil-borne bacteria, fungi and viruses to cause severe damage to the crops (Atkinson, 1892). They predispose some plants to get infected by fungal pathogens.

The approaches for controlling plant parasitic nematodes have been developed primarily for the original nematode population, nematode species and host plant. However, the concept of living with the nematodes' has been strongly supported by the researchers, as eradication of nematode population is impossible and undesirable. Several management approaches such as cultural methods, physical, host resistance, chemical, biological and integrated control methods have been evaluated and recommended for controlling root knot nematodes.

Bernard *et al.* 2017 reported that among biological control on nematode is one of the potential method for management. Under biological control use of nematophagous fungi i.e. nematode killing fungi are found potential option for the ecofriendly management.

Scheid, P.L. (2018) stated that there has been limited exploration of *Stylopage,* among other predatory fungi, as a biological control agent for certain damaging amoebae and nematodes, although no substantive experiments have yet been conducted. The use of *S. araea* in water treatment systems limit the presence of infection-causing prey.

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Samples:

Samples were collected from different selected sites containing decaying wood, decomposed leaves of tree and rhizospheric soils of different places from BHU campus, Varanasi, Uttar Pradesh. 15 samples of fifty gram of composite soil sample from each sites were collected in separate sterile polythene bags, which were double-sealed to prevent evaporation and brought to the laboratory, dried and mixed well from which one gram have been taken for the isolation of desired fungi.

3.2 Observation of Nematophagous Fungi:

For the isolation of Nematophagous fungi, the method used was soil sprinkling method described by Duddington (1955), i.e. Where one gram of sample was sprinkled over the surface of sterile petri dish already poured with sterilized low nutrient agar medium. Population of the pure culture of saprophytic nematode i.e. *Panagrellus redivivus* was added as bait in all of these plates. Three replication of each sample were maintained and incubated at (25±2°C for 15 days).The petri dishes were observed regularly to get the existence of nematophagous fungi under compound microscope as well as light microscope. The fungus were identified and recorded on the basis of formation of trapping structure, observation of formation of spore following the literature provided by several workers time to time (Cooke and Godfrey, 1964; Dayal, 1973; Drechsler, 1935)

3.3 Identification of *Stylopage hadra* and *Acaulopage pectospora* fungi :

For identification of zoophagous fungi *Stylopage hadra* and *Acaulopage pectospora*, spore size, shape were measured along with the observation of trapping structure and compared with the original description given by Drechsler, (1935) and Dayal, (1973).

3.4 Isolation, Purification and maintenance of culture:

Pure culture of fungus was obtained by subsequent transfer of culture to fresh sterilized plates poured with corn meal agar medium (1:10) along with pure culture of saprophytic nematodes added as baits. The culture was transferred from region devoid of contamination to maintain the purity. The culture disc or individual hyphae or mycelium were picked up with the help of sterilized fine needle and placed gently in petri dishes containing corn meal agar medium with saprophytic nematode population and was incubated at 25±2 °C for growth and sporulation. After 5-7 days of inoculation, the culture was retransferred, aseptically into sterilized petri dishes pre-poured with corn meal agar medium (1:10) at 25±2 °C and followed until pure culture obtained. Pure culture of the fungus was maintained by regular and subsequent sub-culturing at the interval of 8-10 days.

3.5 Morphological observation of *Stylopage hadra* and *Acaulopage pectospora* genus:

For morphological observations, culture of *Stylopage hadra* and *Acaulopage pectospora* fungus was grown in Corn meal agar medium (1:10) for growth and sporulation. slides were made in sterilized distilled water from 10-12 day old culture, with profused sporulation, minimum 25 spores of both *Stylopage* and *Acaulopage* were, measured under a research microscope at 40x and 10x magnification respectively. Photographs of spore, hyphae and trapping of saprophytic and plant parasitic nematode were taken in CatCam130-1.3MP microscopic camera.

3.6 Nuclear staining

Nuclear staining of the fungus *Stylopage hadra* and *Acaulopage pectospora* was carried out by using DAPI. Cold Spring Harbor Protocols, given by Chazotte, B. (2011).

1. Dilute the DAPI stock solution 1:5000 in Phosphate buffer saline.
2. Aspirate the cell medium from cells grown on coverslips. Rinse the cells three times with Phosphate buffer saline

3. Fix the cells for 10 min in 3.7% formaldehyde.
4. Aspirate the fixative. Rinse the cells three times, 5 min each, in PBS.
5. Permeabilize the cells by immersion in 0.2% Triton X-100 for 5 min.
6. Aspirate the Triton. Rinse the cells three times, 5 min each, in PBS.
7. Incubate the cells for 1-5 min at room temperature in DAPI labelling solution (from Step 1).
8. Aspirate the labelling solution. Rinse the cells three times in PBS.
9. Mount the coverslips as described in Mounting Live Cells onto Microscope Slides (Chazotte, 2011).
10. Image the cells in fluorescent microscope.

3.7 Molecular characterization and sequencing

3.7.1 Preparation of DNA extraction buffer for isolation of DNA

3M 50 ml stock solution of sodium acetate was made by dissolving 12.30 g of sodium acetate salt in 20 ml of DEPC water and then the pH was adjusted to 5.2 by using 5N sodium hydroxide and at last the final volume of the solution was made to 50ml. A 50 ml 10% SDS stock solution was made by dissolving 5 g of SDS salt in 50 ml of DEPC water. A 0.5 M 50 ml EDTA stock solution was then made by dissolving 7.30 g of EDTA salt in 15 ml of DEPC water and its pH was adjusted to 8.0 by using 5N sodium hydroxide and at last the final volume of the solution was made to 50 ml. The DNA extraction buffer was then made by adding 3.3 ml of 3 M sodium acetate, 1 ml of 0.5 M EDTA and 5 ml of 10% SDS in a 200 ml reagent bottle and mixing gently. 15 ml of DEPC water is then added to the solution and the pH was adjusted to 8.0 by using 5N sodium hydroxide and at last the final volume of the solution was made to 50 ml. The prepared buffer along with the stock solutions was then sterilized in an autoclave at 121°C at 15 lb for 20 min.

3.7.2 Extraction of DNA from fungal mat of *Stylopage* and *Acaulopage* isolate:

The flasks containing sterilized PDB medium were inoculated with 4 to 50.5 mm diameter mycelial bits of fungal culture in the flask under an aseptic condition in a laminar air flow and kept for 8 to 10 days at 28 ± 2°C in an incubator. The fungal mat was harvested after the incubation period and used for DNA extraction as per the protocol described by Patel *et al*. (2016). First of all, 0.2g of the fresh fungal mats were grinded in a mortar and pestle using liquid nitrogen. 0.8 ml of DNA extraction buffer was added to the grind samples and 0.8ml of water-saturated phenol along with 10µml of β-mercaptoethanol was added to the samples while crushing. The samples were then thawed, transferred to Eppendorf tubes and mixed using vortex. The samples were than incubated at room temperature until the mixture turned brown and then centrifuged at 10000 rpm for 5 min. The clear supernatant obtained was than transferred to another Eppendorf tube, 400µml chloroform was added and mixed gently by inverting the tube and then again centrifuged at 10000 rpm for 10 minutes. The upper aqueous layer containing DNA was carefully transferred to fresh Eppendorf tubes without disturbing the lower layers, the 1/3rd volume of 100% ethanol was added to the aqueous layer and kept overnight at 4°C. The samples were than centrifuged at 10000 rpm at 4°C for 20 min and the precipitate was then washed with 70% ethanol. The tubes were then left for drying at room temperature for complete evaporation of ethanol and care was taken not to over dry it. The obtained DNA was then dissolved in 50 µml of nuclease free water. The purity and concentration of DNA were assessed by ratios of *A*260: *A*280 and *A*260: *A*230 using Nanodrop 2000 (Thermo Scientific, Waltham, MA).

3.7.3 Quality assessment of the isolated DNA by Gel Electrophoresis:

First of all, 20 ml of 10X TAE buffer was taken in a 250 ml conical flask and 0.24 g of agarose is dissolved in it by boiling it in a microwave oven until the solution becomes clear to make 1.2% agarose gel solution. The agarose gel solution was allowed to cool down, 1µml of ethidium bromide was added to it (5 µml/100 ml of agarose gel solution), mixed gently and then poured into a gel casting tray with the comb fixed on a plane surface. The comb was removed after the solidification of gel

and was then placed inside an electrophoresis unit filled with 10X TAE buffer. The first well of the gel was loaded with 4 µml of the DNA ladder and subsequently, 3 µml of the DNA samples mixed with 6X gel loading dye was loaded into the subsequent wells. The electrodes were connected to their respective poles and the gel was run for 30 to 40 min at 75V. The gel was then observed in a gel documentation system under the UV light to see the pattern of bands of DNA samples. The primer used in sequencing was ITS1 and ITS4.

Table 3.1: Primer design for Fungus

S. No.	Gene name		Primer Sequence 5' to 3'	Tm	Product size
1	ITS1 and ITS4	F	TCCGTAGGTGAACCTGCGG	54.50	550bp
		R	TCCTCCGCTTATTGATATGC	54.50	550bp

3.7.4 Polymerase chain reaction (PCR) analysis:

The master mix was prepared by mixing 6.5 µml of 10X buffer, 1.0 µml of dNTPs, 0.5 µml of forward primer, 0.5 µml of reverse primer, 0.5 µml of taq-DNA polymerase, and 6.0 µml of DNAse free water for each DNA sample. 10 µml of diluted DNA samples (1000 ng/µml) and 15 µml of master mix was added in a PCR tube. The samples were mixed thoroughly by vortexing for 3 to 5 min. The samples were placed in a thermocycler (BioRad T100) .The PCR amplified product obtained was subjected to agarose gel electrophoresis, for quality confirmation of samples followed by visualization in gel documentation unit.

3.7.5 Molecular identification of fungus using ITS and PCR product sequencing:

Molecular identification of both fungal culture was carried out on the basis of conserved ribosomal inverse transcribed spacer (ITS) region. Amplification was done for the ITS regions between the small nuclear 18S rRNA and large nuclear 28S rRNA using universal primer pairs ITS1 forward (5'-TCCGTA GGTGAACCTGCGG-3') and ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3'.(White et al., 1990). The amplified PCR products of both fungus were sent to Genomics Corp- Xcelris pvt. Ltd. for 18S rRNA partial sequencing. The results of sequencing were provided in FASTA

format. The FASTA sequences of amplified PCR products were used for identification by the procedure described below:

- From the NCBI – BLAST (https://blast.ncbi.nlm.nih.gov/) website, nucleotide blast option was selected.
- FASTA sequence of a sample was placed in query sequence box for *Stylopage* and *Acaulopage* selected respectively in the organism column followed by selecting BLAST option.
- The results obtained after Nucleotide BLAST for both were then analysed on the basis of percent identity and query cover of the samples with closely related species.
- The closely related species showing a high query cover and a high percent identity with the sample were considered as the fungus isolated from the soil confirming the molecular identification of *Stylopage hadra* and *Acaulopage pectospora.*

3.8 Effect of temperature on the growth of *Stylopage hadra* and *Acaulopage pectospora*

Hyphal growth of both fungus viz. *Stylopage hadra* and *Acaulopage pectospora* was studied on corn meal agar medium at different temperature. The media was prepared, autoclaved and poured into several sterilized 90mm petri dishes. 5-7 day old culture was taken and mycelium was picked up with the help of needle and inoculated into petri dishes. The inoculated petri dishes were incubated at different temperature viz.:15°C, 20°C, 25°C and 30°C. Three replications were maintained for each treatment. Hyphal growth of the fungus was measured and recorded daily under stereo compound microscope.

3.9 Effect of media on the growth and sporulation of *Stylopage hadra* and *Acaulopage pectospora*

The hyphal growth of the fungus, *Stylopage hadra* and *Acaulopage pectospora* were studied on eleven different media viz.: Corn Meal Agar medium, Corn Meal Agar medium (1:10), Bark Agar medium, Beef Extract Agar medium, Sabourad Dextrose Agar medium, Richard’s Agar medium, Soil extract Agar

medium, Potato Dextrose Agar medium, Nutrient Agar medium, Yeast Extract Mannitol medium and Martin's Agar medium. Three petri dishes were used for each medium as replicates.

The composition of different media were as follows :

Table 3.2: Composition of different media

Corn Meal Agar Medium		**Beef Extract Agar Medium**	
Corn meal	15.0g	Beef Extract	3.0g
Agar	15.0g	Agar	15.0g
D.W	1000ml	D.W.	1000ml
Corn Meal Agar Medium (1:10)		**Sabourad Dextrose Agar Medium**	
Corn Meal	15.0g	Dextrose	40.0g
Agar	6.0g	Peptone	10.0g
D.W.	1000ml	Agar	20.0g
		D.W.	1000ml
Bark Agar Medium		**Richard's Agar Medium**	
Mango Bark	4.0g	KNO_3	10.0g
Agar	15.0g	K_2HPO_4	5.0g
D.W.	1000ml	$MgSO_4.7H2O$	2.5g
Soil extract Agar Medium		$FeCl_3$	0.02g
Soil	10g	Sucrose	50.0g
Agar	6.0g	D.W.	1000ml
D.W.	1000ml		
Potato Dextrose Agar Medium		**Martin's Agar Medium**	
Potato	250g	Dextrose	10.0g
Dextrose	20.0g	Peptone	5.0g
Agar	20.0g	K_2HPO_4	1.0g
D.W.	1000ml	$MgSO_4.7H_2O$	0.5g
Yeast Extract Mannitol Medium		Agar	15.0g
Yeast extracts	4.0g	D.W.	1000ml
K_2HPO_4	0.5g		

$MgSO_4.7H_2O$	0.2g	**Nutrient Agar Medium**	
NaCl	0.1g	Peptone	5.0g
$CaCO_3$	4.0g	Beef Extract	3.0g
Mannitol	10.0g	NaCl	5.0g
Congo red	2.5ml	Agar	15.0g
Agar	15.0g	D.W.	1000ml
D.W.	1000ml		

All the media were prepared and sterilized in autoclave at 15lbs pressure for 20 minutes. 20 ml of each medium was poured into petri dishes. Individual hyphae or mycelium from 5-7 day old culture were picked, mixes with slightly agar with the help of sterilized fine needle and placed lightly in petri dishes containing corn meal agar medium along with saprophytic nematodes and was incubated at 25±2 °C for growth and sporulation.

3.10 Collection and maintenance of plant parasitic nematode (*Meloidogyne incognita)* and saprophytic nematodes (*Panagrellus redivivus*)

Population of second stage juveniles of *Meloidogyne incognita* was obtained from infected tomato plant and grown in a microplot and regularly maintained in the wire net house of the Department of Mycology and Plant pathology, Institute of Agricultural Sciences, Banaras Hindu University. Sufficient number of egg masses of this nematode was picked from infected roots of tomato and French bean plants and collected separately in a cavity blocks. The cavity blocks containing egg masses were incubated for 4-5 days at 25-28°C for hatching to get required population of 2nd stage juvenile of *Meloidogyne*. Pure culture of saprophytic nematodes was prepared by picking the saprophytic nematodes from the plates having one gram soil sample in already poured water agar medium by sterilized needle bacterial feeder nematode i.e. *Panagrellus redivivus* nematode multiplied and maintained for future use as a bait or experimental purpose.

3.11 Predacity of *Stylopage hadra* and *Acaulopage pectospora* against *M. incognita* and saprophytic nematodes.

25-30 2nd stage juvenile of *Meloidogyne incognita* were picked from the cavity block under stereo microscope and were transferred to separate maize meal agar medium plate to avoid bacterial contamination which might occur due to water droplets which get transferred along with the nematode. From here they were finally transferred in culture plate in close vicinity to the growing hyphae of fungus *Stylopage* and *Acaulopage.*

Nematode transferred were then observed under students compound microscope for observing the predacity of fungus against *Meloidogyne incognita* and saprophytic nematodes. The behaviour of trapping and time of killing was recorded at 25 °C.

CHAPTER 4

RESULTS AND DISCUSSION

4.1) Collection of samples for isolation of Nematophagous fungi:

Samples containing decaying wood, decomposed saw dust from different sites viz., decaying leaf and decomoposed saw dust, decomposed leaf and rhizospheric soil samples of cultivated fields have been collected to isolate different type of nematophagous fungi.

Table 4.1: Colony of nematophagous fungi isolated and identified from different samples

Types of samples collected from different sites	No. of samples	Identified fungus	Number of colony
Decaying leaf and decomposed saw dust	5	*Athrobotrys oligospora* *Drechslerella brochopaga*	1 1
Decomposed leaves	5	*Stylopage* spp. *Monacrosporium eudermatium* *Acaulopage* spp.	2 2 2
Rhizospheric soil of cultivated fields	5	*Arthrobotrys oligospora* *Monacrosporium eudermatium*	2 1

In the experimental sample, *Arthrobotrys oligospora* and *Drechslerella brochopaga* has been dominating from rhizospheric soil and saw dust samples . The occurrence of *Monacrosporium eudermatium* from rhizospheric soils of agricultural crops and that of *Stylopage* and *Acaulopage* spp. have been recorded from decomposed leaf and rhizospheric soil of cultivated fields. The occurrence of *Stylopage* and *Acaulopage* was mostly associated with the sample containing decaying wood and decomposed saw dust soils.

This suggested that the growth of both fungus was highly favoured by natural source of carbon and nitrogen other than the organic chemicals.

4.2) Morphological characterization of *Stylopage hadra* and *Acaulopage pectospora*:

The isolate of *Stylopage hadra* was characterized by mycelium, containing several nuclei in entire protoplasm of hyphae. It sporulate extensively with small (spore characteristics), produced on a short simple straight conidiophore on hyphae. Conidia germinated from single, tapered end with long germ tubes. Spore size was obtained from the Table 5 .The average size of S. hadra ranged from 22.5-35µm long and 11.5-17.5µm in width while the size of A. pectosporaspore ranged from 162.5-237.5µm long and followed by width 4-5µm. (Plate-1 for *S. hadra*; Plate- 3.1 for *A. pectospora*). Acaulopage was characterized by coenocytic hyphae that have patches of yellowish adhesive patches used to capture amoeba.Conidia are borne directly on the hyphae and vary widely in morphology. Many bear one to many empty appendages.

Similar observation regarding the morphology, conidia size were recorded by Drechsler (1935) and Das-Gupta S.N. (1996) for *Stylopage hadra* and *A. pectospora* respectively.

Table 4.2: Spore measurement of *Stylopage* spp. and *Acaulopage* spp.

S.no.	Spore dimension of *Stylopage hadra* at 40X magnification		Spore dimension of *Acaulopage pectospora* at 40X magnnification	
	Length (μm)	Width(μm)	Length(μm)	Width(μm)
1	27.5	12.5	187.5	4.5
2	25	12.5	215	4
3	28.1	15	180	5
4	25.6	13.1	162.5	4.8
5	27.5	12.5	175	5
6	25	12.5	192.5	5
7	27.8	15	200	4.7
8	27.5	15.3	170	4.2
9	26.2	2.5	237.5	4
10	25	12.1	225	4.2
11	27.5	17.2	180	5
12	33.1	17.2	170	4.5
13	31.2	13.7	200	4.8
14	22.5	11.2	190	4.5
15	30	13.7	175	4.6
16	32.7	17.2	200	5
17	35	17.5	200	4.6
18	30	12.5	162.5	4.5
19	25	12.5	200	5
20	28.7	13.1	180	5
21	25.6	12.5	190	4.5
22	25	13.2	185	4.7
23	28.7	14.2	200	4.4
24	30	13.7	225	4.5
25	25	12.2	200	4.8
Avg.	27.8	13.8	191.5	4.6
Max.	35	17.5	237.5	5
Min.	22.5	11.2	162.5	4

4.3) Nuclear staining

4', 6-diamidino-2-phenylindole staining was used to visualized location and number of nuclei in living cell and fixed cell. *Acaulopage* and *Stylopage* with DAPI showed no ultrastructural changes compared to the appearance of original fungal cells. DAPI staining is only used for appearance of nuclei and staining allows multiple uses of cells eliminating the need for duplicate samples.

After staining of *Acaulopage* and *Stylopage* with DAPI it was found that a coenocytic hyphae functions as a single coordinated unit composed of multiple cells linked structurally and functionally in fungal mycelium (Plate -5).Selected strains of nematophagous fungi shows coenocytic type mycelium and have more than one nuclei and randomly scattered nuclei in mycelium.

4.4) Molecular Characterization

Molecular identifications of selected isolates were done by 18S rRNA gene sequencing analysis. The partial 18S rRNA sequences was compared with the sequences available at Gene Bank, EMBL and DJB databases using nucleotide BLASTn 2.3.21 program through NCBI server. 18S rRNA partial sequenced was done by Genomic corp xcelris lab pvt ltd. The fungal isolate *Stylopage hadra* showed 93 percent identity with *Stylopage hadra* and *Acaulopage pectospora* exhibited 97 percent identity with *Zoophagus pectosporus*

The results were found to be similar with the findings of Davis *et. al.,*(2019) and Corsaro *et. al.,* (2018).

- ***Stylopage hadra* with ITS sequencing**

CAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTAATAG
CTTTCCAGGCGATCCGGGAATTATCGGATATCAGTTATAGTTTATTTGATGT

TACCTACTACTAGGATATCCGTGGTAATTCTAGAGCTAATACTTGCACAAAG
GCCCGACTTCAGGAAGGGCGGTATTTATTAGTTAAAAACCAACCCGGGCAA
CCGGAACCTTGGTGATTCATAGTAACTTTACGAATTGCATGGCTTCATGCCG
GCAATGGTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAG
GCCTACCATGGTGACAACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGA
GGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAA
ATTACCCAATCCCAATACGGGGAGGTAGTGACGAAAAATAACAATACAGG
GCCATAAGGTCTTGTAATTGGAATGAGCACAATTTAAAATCCTTAGCAAGG
AACCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAA
GTTGTTGCAGTTAAAAAGCTCGTAGTCGAATTTTGGGGTTTGGGCTGGTGGT
CCATCGTTTCGGTGCGTACTGCCGAGCCCGGCTCTTTTTCCTGGGGATCTGC
CGGACAGCCGCAAGGCTGCTCGGTAGCGAGCCAGGGTGTTTACCTTGAAAA
ATTGGAGGGCAAGTAAATTAGAGTGCTTAAAGCAGGCATTAAGCTTGAATA
TATTAGCATGGAATAACTGAATAGGACTCTGGTCTTATTTTGTTGGTTTAAG
GGGCTGGGGTAATGGTTAATAGGAACAGTCGGGGACATTTGAATTGCTGAG
CTAGAGGTGAAATTCTTGGATTTCAGCAAGTCAAACTAAGGCGAAAGCATT
TGTCAAAAACGGGTCATTGATCAAGAACGAAAGTTAAGGGATCAAAGACG
ATCAGATACCGTCGTAGTCTTAACCGTAAACTATGCCGACTGGAGATTGGG
CTAAGTTGCTTATGACTTGCCCAGCATCACTTCGAGAAATCAAAGTTTTTGG
GTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAA
GGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAA
ACTCACCAGGTCCAGACATAGTAAGGACTGACAGATTGAAGCTCTTTCTTG
ATTCTATGGTTGGTGGTGCATGGCCGTTGCTAGTTCGTGGAGTGATTTGTCT
GGTTAATTCCGATAACGGACGAGACCGTAATCTTTAACTAGTGAGACTCATT
TTATGATGAGCTGACCACTTCTTAAGGAGACGCTAGATTCTTAATCTACTGA
AGTACGGCTCTAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCG
CGCTACACTGACAGAGCCAGCGAGTCTATCCTAAACCGAAAGGTTCTGGGA
AATCTGGCAAACTCTGTCGTGATGGGGATAGTTCATTGCAATTATTGAACTT
GAACGAGGAATTCCTAGTAAGCGCACCAGGAGTGGAGCCTGCGGCTTAATT
TGACTCAACACGGGAAAACTCACCAGGTCCAGACATAGTAAGGACTGTATG
GTTGGTGGTGCATGGCCGTTGCTAGTTCGTGGAGTGATTTGTACAGATTGAA

GCTCTTTCTTGATTCCTGGTTAATTCCGATAACGGACGAGACCGTAATCTTT
AAATAGC

- ***Acaulopage pectospora* with ITS sequencing**

AAACTGCGAACGGCTCATTATATCAGTTATAGTTTATTTGATGGTACCTTA
CCACTAGGATATCCGTGGTAATTCTAGAGCTAATACTTGCACAAAATCCA
AAACTCGGGTAAGGCTTCTTTATTAGTTAAAAACCAACCCGGGCAACCGG
AATTTTGGTATTCATAGTGGAGTGGAGCCTGCGGCTTTGACTCAACACGG
GAAAACTCACCAGGTCCAGACATAGTAAGGACTGACAGATTGAAGCTCG
AGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCA
ATCCCAATTCGGGGAGGTAGTGACGAAAAATAACAATACAGGGCCAATA
GGTCTTGTAATTGGAATGAGCACAATTTTAGCAAGGAACAATTGGAGGGC
CTTTACGAATCGCATGGCTTCACGCCGGCGATGGTTCATTCAAATTTCTGC
CCTAAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTAT
ATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTCGAATTTTGGGGCTGAGG
CGGGCGGTCTGCCGTTTCGGCCTCGCCTGGCTCTTTTTCCTGGGGAACTGT
TGGGTCGTCGCAAGGCGGCGTGGCAGCAAGCCAGGGTGTTTACCTTGAAA
AAATTAGAGTGTTTAAAGCAGGCGCAAGCTTGAATATATTAGCATGGAAT
AACTGAATAGGACTTTGGTCTTATTTTGTTGGTTTAAGAGACCAAGGTAAT
GGTTAATAGGAACAGTCGGGGACATTTGAATTGCTGGTTTTCATTGATCAA
GAACGAAAGTTAAGGGATCAAAGACGATCAGATACCGTCGTAGTCTTAAC
CGTAAACTATGCCGACTGGAGATTGGGCAAAGTTGCTTATGACTTGCTCA
GCATCCCACCGAGAAATCCAAGTTTCTGGGTTCTGGGGGGAGTATGGTCG
CAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCAAGTCATCAGCTT
GCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTTCTGA
TTAGGGATCCCAGTGAGGACTTTGGATTGCTAGCGGGTGCTGGAAACGGT
AACCTAGCTCGCGAGAAGCTGTTCAAACTTGGTCATTTAGAGGAAGTAAA
AGTCGTAACAAG

4.5) Analysing hyphal growth of *Stylopage hadra* and *Acaulopage pectospora* on day to day basis:

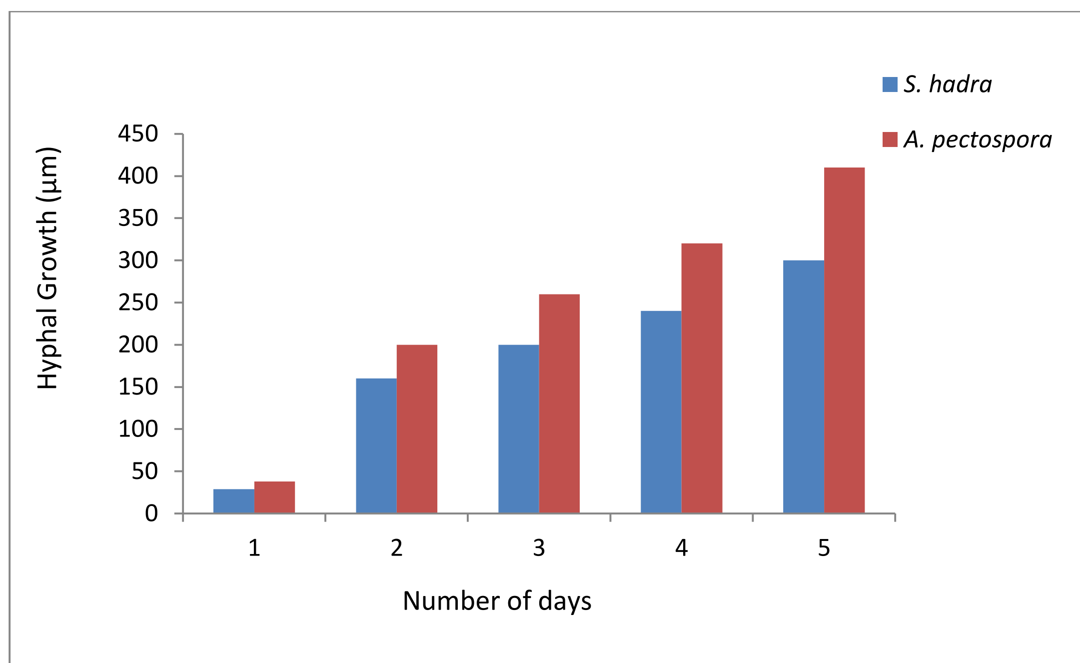

Figure 4.1: Analysing hyphal growth of *Stylopage hadra* and *Acaulopage pectospora* on day to day basis.

Hyphal growth of St*ylopage hadra* and *Acaulopage pectospora* on corn meal agar medium was watched carefully for 5 days on daily observation basis. The hyphal growth of *Stylopage hadra* and *Acaulopage pectospora* showed consistent increase in hyphal growth with increasing number of days. The maximum growth for *Stylopage hadra* was recorded on fifth day as 240µm whereas 380µm was recorded for *Acauloapge pectospora.* The minimum hyphal growth was recorded on the first day as 29µm for *Stylopage hadra* and 35µm for *Acaulopage pectospora.*

Thus *Acaulopage pectospora* showed higher and fast hyphal growth than *Stylopage hadra* when observed and recorded carefully at 25°C on maize meal agar medium as displayed by Fig. 4.1

4.6) Effect of different temperature on the hyphal growth of *Stylopage hadra* and *Acaulopage pectospora* (after 5 days).

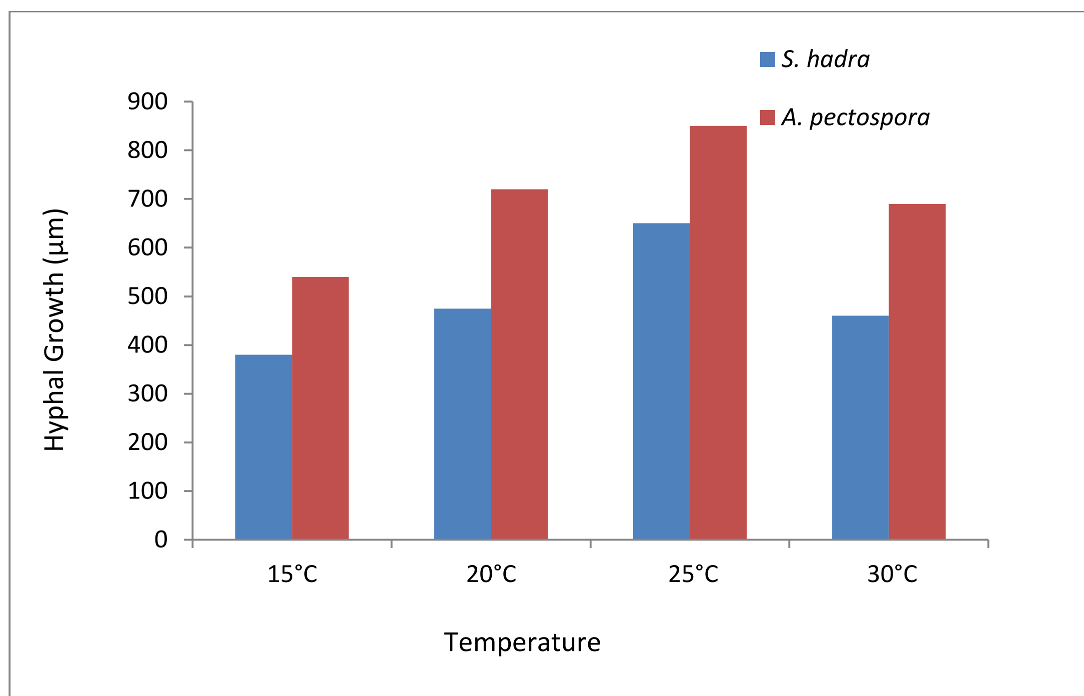

Figure 4.2: Effect of different temperature on the hyphal growth of *Stylopage hadra* and *Acaulopage Pectospora*(after 5 days).

Hyphal Growth of *Stylopag*e and *Acaulopage* was significantly varied when studied with different temperature on corn meal agar medium. The observation of hyphal growth recorded after 5 days of inoculation. *Stylopage* recorded maximum growth as 650 µm after 5 days at 25°C whereas maximum growth of *Acaulopage* was also recorded at similar temperature as 850 micrometre. The mimimum hyphal growth after 5 days was recorded as 350 µm for *Stylopage* while 450 µm for *Acaulopag*e at 15°C. Both fungal species showed optimum growth at 25°C while less sporulation were recorded at 20°C and 30°C well depicted wit Fig 4.2

4.7) Effect of different media on hyphal growth of *Stylopage hadra* and *Acaulopage pectospora*

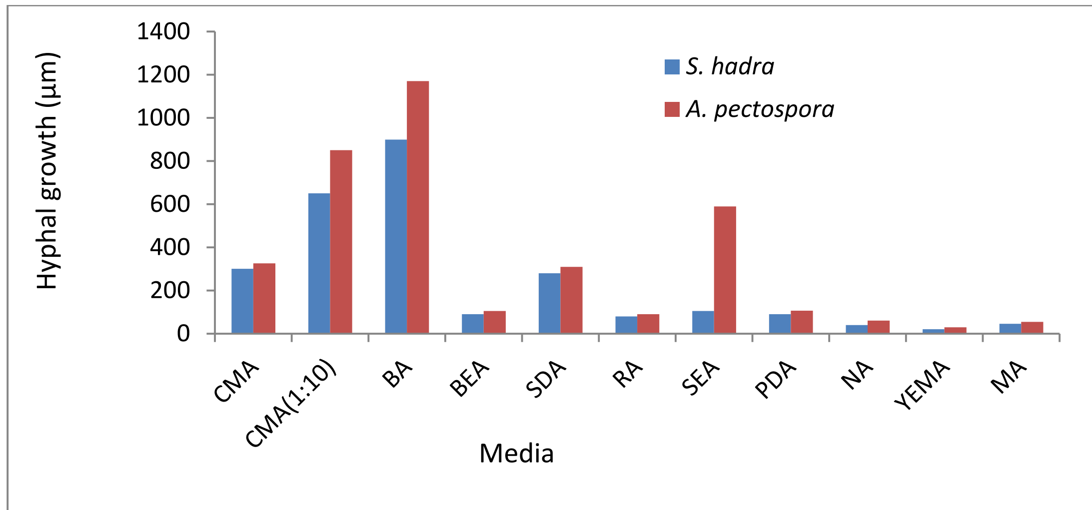

Figure 4.3: Effect of different media on hyphal growth of *Stylopage hadra* and *Acaulopage pectospora* at 25 ° C

The growth and sporulation of *Stylopage* and *Acaulopag*e were studied on different culture media at 25°C. The growth of both fungi was highly favoured by natural source of carbon and nitrogen other than the organic chemicals. The varying growth pattern and sporulation behaviour of *Stylopage* and *Acaulopage* were significantly varied on different media for their nutritional requirements.

Maximum growth along with intensive sporulation was recorded on Mango bark agar medium, The hyphal growth after 5 days was recorded as 850 µm in *Stylopage* and 1250 µm in *Acaulopage*, while yeast extract mannitol agar medium recorded minimum growth with no sporulation due to inhibition of fungal growth by bacterial contamination.

After Bark agar medium highest growth of both fungi was recorded in Corn meal agar medium (1:10) with *Stylopage* hyphal growth reaching 650 µm and *Acaulopage* hyphal growth recorded as 820 µm with delayed but made good sporulation, witnessing few germinated spore with one end in *Stylopage* and with one or both ends in *Acaulopage*, followed by Soil extract agar medium recorded 150µm hyphal growth for *Stylopage hadra* and 450µm hyphal growth for *Acaulopage*

pectospora suggesting to be a preferable media over other media used to assess the growth of both fungi.

The fungus *Stylopage* and *Acaulopage* seems to be quite specific to nutrition as they recorded profused growth over only three media viz.Mango bark agar, corn meal agar medium(1:10) and corn meal agar medium. Other medium recorded less or minimum hyphal growth and covered by bacterial growth which didn't allows/ is somewhat obligate to the fungus to grow well as depicted in Fig 4.3. Since the fungus *Stylopage* and *Acaulopage* requires bacterium free medium along with good number of sapropohytic nematodes for their hyphal growth and sporulation. Higher number of nematode killing provides fastidious growth along with preferable nutritional sources. Solid medium provides lesser opportunities for hyphal growth and sporulation in comparison to semi solid media such as corn meal agar (1:10) media recorded much higher hyphal growth after 5 days for both fungus when compared with corn meal agar medium.

4.8) Predacity of *Stylopage hadra* and *Acaulopage pectospora* on saprophytic and plant parasitic nematode

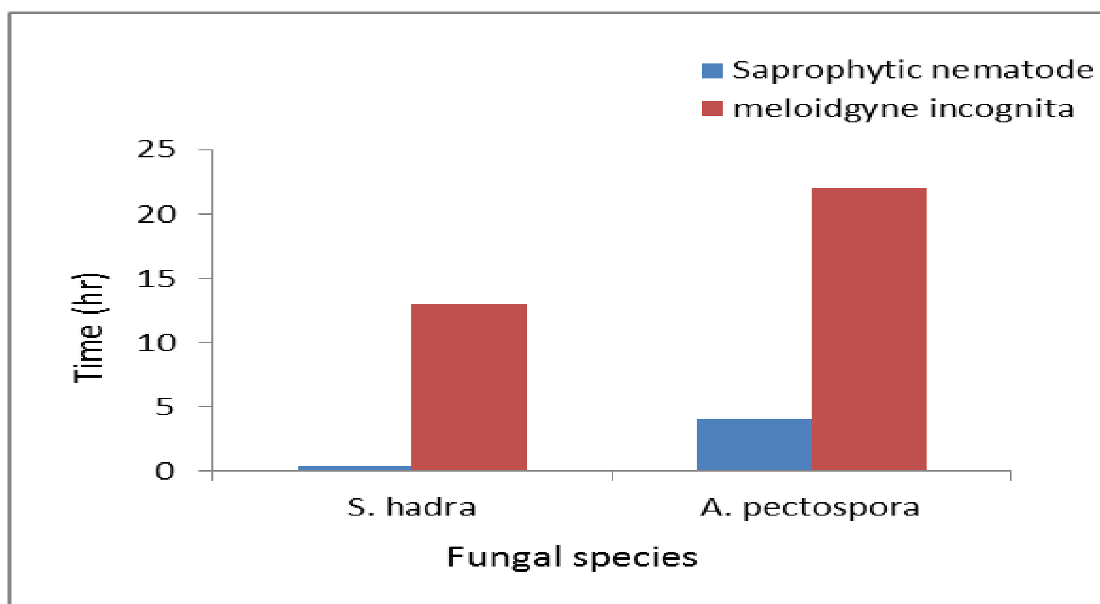

Figure 4.4: Predacity of Stylopage hadra and Acaulopage pectospora on Meloidgyne spp. and saprophytic nematode

Observation on predacity of *Stylolpage* and *Acaulopage* (PLATE-2 for *S. hadra;* PLATE-4 for *A. pectospora*) against the plant parasitic and saprophytic nematode shows greater degree of variance in their behaviour of trapping and killing. The inducement of trapping structure formation and predatory ability is also influenced by total number of interacting nematodes. Higher nematode count facilitates fastidious growth with more number of killing rate.

Stylopage traps the nematode with adhesive hyphae, entire hyphae behaves as a sticky hyphae which the nematode gets attached at every point wherever it interacts whereas in *Acaulopage t*he killing is characterized with a raised hyphal stalk like structure with which the nematode gets attached. These stalks are arranged in an alternate fashion with a distance of 0.4-25µm thus providing lesser contact chances for nematodes and leading to delayed killing as represented by Figure 4.4.

The fungus *Stylopage* recorded nearly 0.4hr time (15 minutes) from trapping to killing of a nematode whereas it was recorded to nearly 4 hr time (240 min), almost 15 times more than what taken by *Stylopage* suggesting higher predacity of *Stylopage* against saprophytic nematodes.

The fungus *Stylopage* recorded nearly 13 hour time from trapping to killing of *Meloidgyne incognita*, whereas the fungus *Acaulopage* recorded 22 hour time from trapping to killing of the nematode. Almost double time taken by the fungus *Stylopag*e as compare to *Acaulopage*. According to result the fungus *Stylopage* has a higher degree of predacity against both nematodes when compared to *Acaulopage*.

CHAPTER 5

SUMMARY AND CONCLUSION

Nematophagous fungi are cosmopolitan microorganisms able to modify their saprophytic behaviour to carnivorous, allowing them to feed on nematodes under unfavourable nutritional conditions. They are natural enemies of nematodes and have developed highly sophisticated strategies of infection. There are over 700 nematophagous fungal species, from several phyla, such as the Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota. Moreover, even organisms belonging to the phylum Oomycota have had their nematophagous activity. Traditionally, these fungi are classified into three groups according to their characteristics of predation on nematodes: (1) nematode-trapping (2) opportunistic or ovicidal and (3) endoparasitic fungi. Most of the fungi belonging to Zygomycota are known for its predator to amoeba few of them also reported to kill nematode. Among them species of *Stylopage* and *Acaulopage* belongs to closely related genera. It was difficult to isolate both of these fungi due to their life system and to culture them. When both the fungi *Stylopage* and *Acaulopage* were isolated from different sites their frequency were recorded very low as compare to other nematophgous fungi belonging to higher group of fungi pre-dominated by Hyphomyetes. Both the fungi *Stylopage hadra* and *Acaulopage pectopsora* were identified first on the basis of morphological characterization on the basis of conidia shape and size their characteristic means of killing nematodes by adhesive hyphae with slightly globose protuberance at the site of attachment of the nematodes on the basis of its identification key provided by some researchers. Both the fungus were obtained either from decomposed leaves or rhizospheric soil indicating that the growth of both fungus was highly favoured by natural source of carbon and nitrogen other than the organic chemicals. Average size of conidia in Stylopage hadra was found 27.8µm length and 13.8µm width whereas 191.5µm length and 4.6µm width for *Acaulopage pectospora* along with coenoctic mycelium which was determined by Nuclear staining of hyphae of both the fungi under Fluorescent microscope where nucleus in the hyphae moved freely in the protoplasm of hyphae as in the aseptate hyphae. Hyphae

of *A. pectospora* was found with alternate raise perpendicular stalks arranged in a random alternate manner. After molecular characterization the fungal isolate *Stylopage hadra* showed 93 percent identity with *Styloage hadra* and *Acaulopage pectospora* exhibited 97 percent identity with *Zoophagus pectosporus* when sequenced.

When both the fungi assessed for their growth and sporulation at different temperature *A. pectospora* showed higher and fast hyphal growth than *S. hadra* when observed and recorded on maize meal agar medium while both the fungus species showed optimum growth at 25°C with less sporulation recorded at 20°C and 30°C. On different media used for their growth maximum growth of both the fungus along with intensive sporulation was recorded on Mango bark agar medium followed by corn meal agar medium(1:10) and it was observed that faster growth on semi-solid media rather than solid media. The fungus *Stylopage* and *Acaulopage* seems to be quite specific to nutritional requirement as they recorded profused growth over selected media. Both the fungus *S. hadra* and *A. pectospora* requires bacterium free medium along with good number of sapropohytic nematodes for their hyphal growth and sporulation. Fungus *Stylopage* and *Acaulopage* requires bacterium free medium along with good number of sapropohytic nematodes for their hyphal growth and sporulation chances for nematodes with *A. pectospora* and leading to delayed killing.

When both the fungi tested for their predacity with saprophytic and plant parasitic nematodes i.e *Meloidogyne incognita*, large number of saprophytic and plant parasitic nematodes found trapped with *S. hadra* than *A. pectospora* while killing time was recorded more with *A. pectospora* than *S. hadra* for both saprophytic and plant parasitic nematodes.

In view of this from this research work it may be concluded that both the fungi generally known for capturing and killing amoebae but its predacity potential for both saprophytic and plant parasitic nematodes may be explored in the future to utilize these fungi for a possible biocontrol agent for the management of root knot disease in different crops.

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